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Functional studies on the shell soluble matrix of *Anodonta cygnea* (Bivalvia: Unionidae)

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ABSTRACT

The biomineralization process in molluscan shells is controlled by an extracellular organic matrix, embedded in a fluid, produced by the calcifying outer mantle epithelium (OME) and secreted within the extrapallial compartment.

In the present work, the study subject is the nacreous layer of the freshwater bivalve *Anodonta cygnea* and the functional role of its organic matrix, which is still a large field to explore. From the organic matrix it was possible to extract two fractions, but only the soluble fraction was studied. Different techniques were used, including biochemical protein characterization by electrophoresis for the extrapallial fluid and the shell, quantification and detection of the matrix proteins and glycosamynoglycans (GAGs) directly in the shell, through immunogold techniques, using SEM and ATR-IR observations. Seven protein fractions in both extrapallial fluid and shell were detected by electrophoretic analysis with molecular weights of approximately 102/106, 76/74, 66/66, 60/52, 45/43, 35/35 and 31/29 KDa, respectively. This may suggest a narrow functional correlation between specific proteins from the extrapallial fluid and the shell. Despite the low percentage of the organic matrix relative to the whole nacreous shell, it was observed that it is mainly composed of proteins (13.40–23.32 mg/ml) and GAGs (2.50–3.12 mg/ml), which appear to be very relevant on the microstructure and polymorphism organization of the major calcium carbonate fraction. In agreement, the immunogold technique showed that the shell organic matrix is mainly intercrystalline. Additionally, the common detection by infrared spectroscopy of amide groups on both soluble shell matrix and solid shell crystal fraction suggests that this molecule is one of the intracrystalline inductors of the aragonite crystals formation in the nacreous layer of *A. cygnea*.

Additional Keywords: biomineralization, extrapallial fluids, organic matrix

INTRODUCTION

The shell structure in the molluscan shell carries a good historical record that helps explain the evolution of this

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group of animals since the Cambrian period. Shells are secreted by a vast majority of the estimated 70,000–76,000 named molluscan species (estimate in Rosenberg, 2014) and their construction begins in the early stages of development and almost continuously during their entire life (Bøggild, 1930; Marin and Luquet, 2004). The shell crystals in bivalves show a great variety of morphologies and organization levels originating different microstructures and polymorphisms (Checa et al., 2007; Lopes-Lima et al., 2010). However, all of them are constituted by calcium carbonate representing 95–99% of the shell and by 1–5% corresponding to organic matrix (Duplat et al., 2006). Adult shells are highly variable being built up of one or more shell layers, each of which may have a different microstructure (Bøggild, 1930; Weiss et al. 2002). In a longitudinal section, the Unionidae shell reveals generally two calcified layers, prismatic and nacreous layers, and one outer organic layer, the periostracum, which protects the calcified layers from water dissolution (Bøggild, 1930; Moura et al. 2003; Marin and Luquet, 2004). The most common mineral polymorphs identified in the calcified layers of calcium carbonate are aragonite and/or calcite (Weiner, 1983; Checa et al., 2007). In the families Pinnidae and Pteriidae the shell has one internal layer with calcium carbonate in the aragonite form and another external in the calcite form whereas in the Unionidae family the two calcified layers are both aragonitic (Bøggild, 1930; Taylor et al., 1969; Caiping et al., 2005; Marie et al., 2007).

Although the shell calcification process occurs outside the living tissues, it is neither in contact nor directly dependent on the external environment. The process is mainly dependent on three components: a closed compartment where the calcification occurs, an ionic membrane transport and an extracellular organic matrix (Moura et al., 2003). The closed compartment filled with extrapallial fluid is bounded by the shell, the periostracum, and the calcifying outer mantle epithelium (OME). This isolation is critical to provide a supersaturated environment which is essential for the formation of crystals (Marin and Luquet, 2004). The extracellular organic matrix, secreted by the calcifying epithelium towards the extrapallial fluid, consists of a complex mixture of proteins, glycoproteins, proteoglycans and chitin (Moura, 2000; Pereira-Mouriès et al., 2002). This matrix has important roles in the physical-chemical interactions involved in crystal nucleation, polymorphic seleetion, growth, and inhibition (Marxen and Becker, 1997; Levi-Kalisman et al., 2001; Pereira-Mouriès et al., 2002).

Little is known about the extracellular organic matrix of the unionid freshwater mussel *Anodonta cygnea*. The shell exhibits the three layers already mentioned and, though the two calcified layers present different microstructures, both correspond to the same calcium carbonate polymorph, aragonite. According to Moura et al. (2000) there are 4–6 protein fractions in the calcifying fluids of *A. cygnea*.

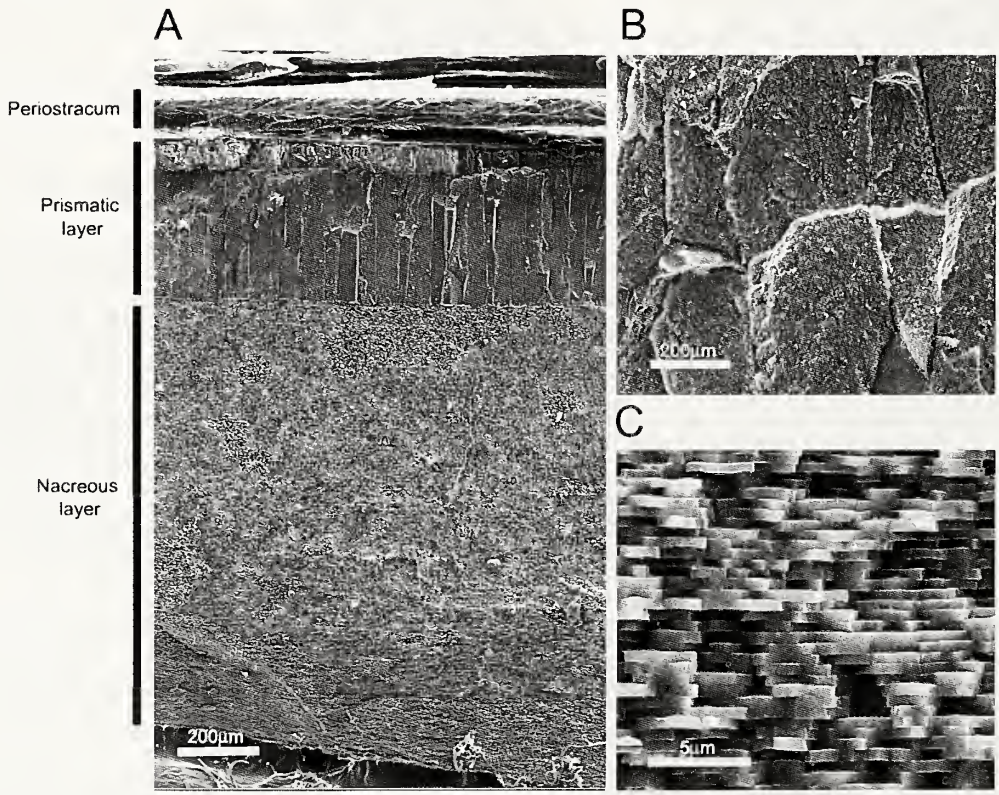

Figure 1. SEM images of the shell of *Anodonta cygnea* in back-scattered electron mode. (A) The three layers are perfectly distinct in transversal sections; magnification of prismatic (B) and nacreous (C) layers.

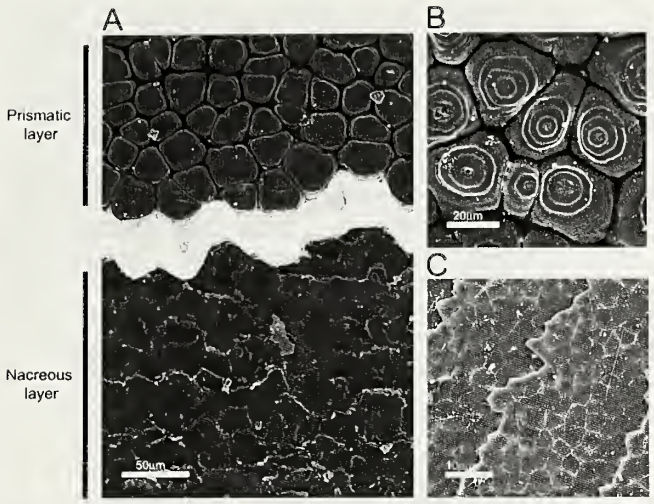

Figure 2. SEM images of the external shell surfaces of *Anodonta cygnea* in back-scattered electron mode (A). Magnification of prismatic (B) and nacreous (C) layers.

The objective of this work is to study the soluble fraction of the extracellular organic matrix from the extrapallial fluid and nacreous layer of the freshwater mussel *Anodonta cygnea,* in order to gain further knowledge about its components. The main goal was to extract and purify the organic matrix from the shell in order to quantify and analyze it by electrophoresis and Attenuated Total Reflectance-Infrared Spectroscopy (ATR-IR). The direct visualization of the organic matrix presence in the shell was also an aim of the study, through the use of immunohistochemical techniques.

MATERIALS AND METHODS

Organic Matrix Extraction, Quantification and SDS-PAGE Analysis

Freshwater bivalves, *Anodonta cygnea*, were collected from the bottom of Mira Lagoon (40°26.712N, 8°47.817W) in the end of July 2012. The nacreous layer organic matrix was extracted as described by Caiping et al. (2005) with the following exceptions: the initial amount of nacreous layer sample was higher (90 g), the dialysis was performed against ultrapure water and, at the end, the sample powder was re-dissolved in PBS buffer.

The TCA-DOC protein precipitation technique was used for the extraction of very low contents of soluble protein from the extrapallial fluid. For this, to the extrapallial fluid, was added 1/100 vol. of 2% DOC (sodium deoxycholate), followed by the addition of 1/10 of trichloroacetic acid (TCA) 100% and centrifuged for 15 min at 4 °C in a microfuge at maximum speed (15000 g). For the SDS-PAGE, the protein pellet was re-suspended in a minimal volume in PBS buffer.

In this work only the soluble fraction was analyzed. Two independent replicas of the extraction were accomplished. Total protein and glycosaminoglycans concentrations in the shell were determined respectively according to the methods of Bradford (1979) and Whiteman (1973). Protein fractions from the extrapallial fluid and shell samples and molecular weight standard were separated and analyzed using a mini-SDS-PAGE system in 8% polyacrylamide gels at 130V during 60 min and stained with silver nitrate (Gromova and Celis, 2006).

Peptide Mass Mapping from the Extrapallial Fluid and Shell Protein Band

The observed protein band from the SDS-PAGE gel was cut and transferred to the Eppendorf tubes before being sent to Alphalyse, Inc. (USA) for peptide mass mapping. From the seven protein bands detected, only the five marked bands with highest expression were sent for analysis (Figure 4). The protein samples were reduced and alkylated with iodoacetamide, i.e., carbamidomethylated, and subsequently digested with trypsin and chymotrypsin. Trypsin cleaves after lysine and arginine residues. The resulting peptides were spotted directly onto an anchorchip target or were concentrated on a C18 ZipTip micropurification column and eluted onto an anchorchip target for analysis on a Bruker Autoflex Speed MALDI TOF/TOF instrument. The peptide mixture was analyzed in positive reflector mode for accurate peptide mass determination (MALDI-MS).

Polyclonal Antibodies Production and Visualization of the Shell Organic Matrix by Immunogold Technique

The polyclonal antibodies were produced in two rabbits. For each immunization 100 µg of organic matrix were injected in the intradermic neck region. The immunization procedures were reinforced after 30 and 42 days. Bleedings were carried at 0 (pre-immune serum), 30, 42, and 54 days. The sera were then titrated by standard ELISA assays, for evaluation of the more appropriate antibody concentration. The third bleeding was then chosen and used in the following procedures. IgGs were purified in a protein G column (GE Healthcare) and used in the immunogold assays. This assay was performed as described by Marin et al. (2007). Briefly, the

Table 1 Quantitative results of organic matrix extraction from the nacreous layer of *A. cygnea*.

Sample	Initial shell (g)	Final organic matrix powder (g)	Organic matrix % (w/w)	Total proteins (mg/ml)	Total GAGs (mg/ml)
1	90	0,0347	0,039	23,32 ± 0,27	3,12 ± 0,11
2	90	0,0476	0,053	13,40 ± 0,35	2,05 ± 0,03

nacreous layer was broken in small pieces and etched with EDTA 1% (w/v), pH 7.5 during 2-3 min with agitation. The pieces were then incubated overnight with a 1:3000 dilution of the IgGs produced against the organic matrix. The secondary antibody used (Anti-rabbit IgG – Gold antibody produced in goat, affinity isolated antibody, aqueous glycerol suspension, 5 nm, Sigma) was diluted 1:400 and incubated during 2 h. The silver enhancement was performed with a Silver Enhancer Kit by Sigma. As a negative control the pieces were first incubated with pre-immune serum. The results were observed through scanning electron microscopy (SEM).

Scanning Electron Microscopy Imaging (SEM) of the Shell and Attenuated Total Reflectance-Infrared Spectroscopy (ATR-IR) of Aqueous Shell Matrix.

Untreated shell pieces were gold-coated (FINE-COAT Ion sputter JFC-1100) and glued to aluminum stubs for SEM observations using JEOL JSM-35C scanning electron microscope operated at 15 keV in Centro de Materiais da Universidade do Porto (CEMUP). Shell pieces treated with immunogold technique were carbon-coated and analyzed in back-scattered electron mode at 15 keV.

Two aqueous samples of soluble organic matrix were analyzed by ATR-IR using a Bruker Tensor-27 spectrometer equipped with a DTGS (deuterated triglycine sulfate) single detector plate and a horizontal ATR unit, where a horizontal ZnSe ATR crystal was mounted at 45° in a 30 ml rectangular cell made of polypropylene. Samples were run in the frequency range 800–4000 cm^{-1}. Additionally, few milligrams of nacreous shell layer were extracted from the freshwater mussel *A. cygnea* and then were analyzed by infrared spectroscopy in absorbance mode using a BRUKER Tensor-45 spectrometer. The pellet disks of 1.5 cm diameter were prepared by mixing 1 mg of sample with 200 mg KBr and pressing at 10 Kg/cm^2.

RESULTS AND DISCUSSION

Characterization of the Natural Microstructure of *Anodonta cygnea*

SEM observations (Figures 1 and 2) highlight the nature of the shell microstructure in the freshwater mussel *A. cygnea*. The three different layers are evident: one organic (periostracum) and two calcareous layers (prismatic and nacreous). In the prismatic layer the aragonitic crystals are organized in prisms covered with organic matrix surrounding them, whereas in the nacreous layer the crystals are organized with the shape of tablets with organic matrix between them.

In this work, we have mainly focused on the nacreous layer, specifically in its organic matrix. Regarding the shell nacreous layer, the quantitative results of its main organic components are presented in Table 1. As described in the literature for *A. cygnea* by Moura et al.

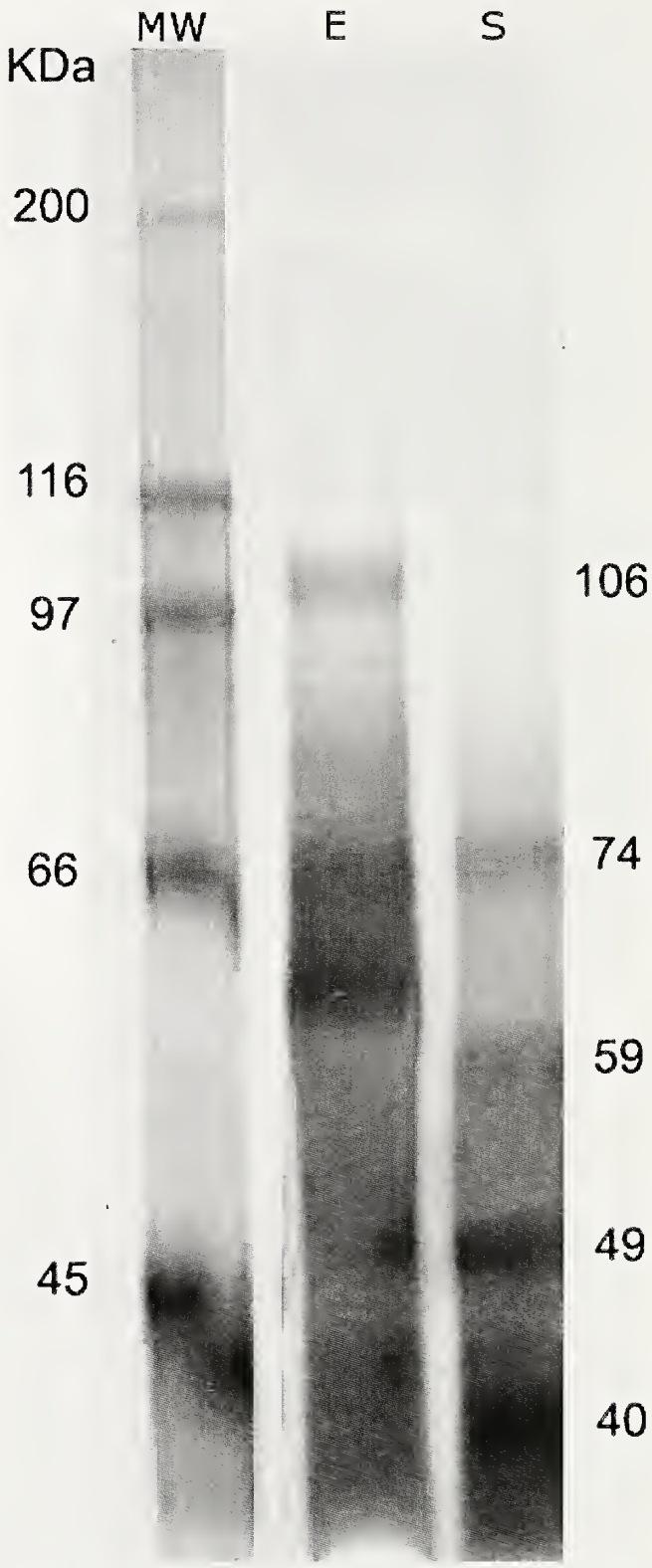

Figure 3. SDS-PAGE of the soluble organic matrix extracted from the extrapallial fluid (E) and shell nacreous layer (S) of *Anodonta cygnea*. MW: molecular weight standards; E: Protein fraction from the extrapallial fluid; S: Protein fraction from the shell.

(2000) and for other species of bivalves (Marie et al., 2007), it was also stated that the organic matrix represents a small amount of the total shell weight. The majority of this matrix is represented by proteins (13.40–23.32 $mg.ml^{-1}$), while the glycosaminoglycans (GAGs) were found in lesser amounts (2.50–3.12 $mg.ml^{-1}$). Although present in smaller amounts, GAGs are always found in the organic matrix, denoting their importance in most biomineralizing systems (Pereira-Mouriès et al., 2002; Moura et al., 2000, 2003; Lopes-Lima et al., 2005, 2010). Naturally, the total protein and GAGs contents were higher in the shell matrix compared to the organic fluids as reported by Moura et al. (2000) for the same period. In fact, these results confirm that the shell matrix structure act as a sponge. Furthermore, while results of Moura et al. (2000) reported 6 protein fractions on the haemolymph and extrapallial fluids along the year, the present study adds complementary data based on the detection of seven different protein bands in extrapallial fluid which are similar to others in the shell matrix of *A. cygnea* (Figure 3).

The SDS-PAGE technique recorded seven protein fractions with molecular weights bands of approximately 102-106, 76-74, 66-66, 60-52, 45-43, 35-35, and 31-29 KDa,

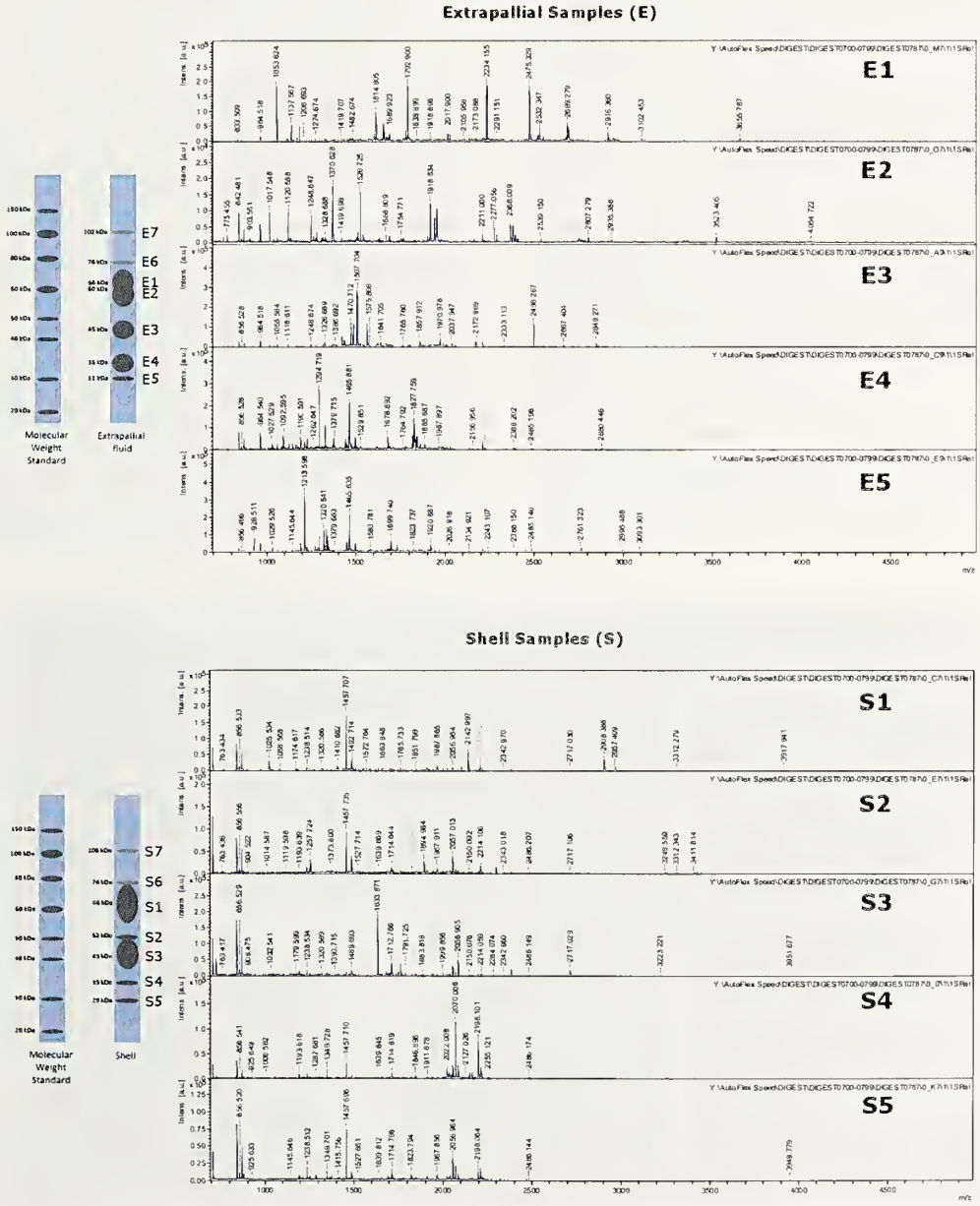

Figure 4. Diagram of a spectra of peptides mass mapping of soluble organic matrix extracted from the extrapallial fluid (E1-E5) and shell nacreous layer (S1-S5) of *A. cygnea* by MALDI-MS determination.

respectively in the extrapallial fluids and in the shell matrix (Figure 3). However, from these seven bands, only five were submitted for peptide mass analysis, since the two heaviest (74-106 KDa) showed poor resolution. According to Moura et al. (2000), the protein bands in the fluids can show different expression levels along the year, which can be correlated with its own functional role in the shell. Similar studies by electrophoresis have been previously performed in at least three families of mollusks, two of them in the Bivalvia, and the number of protein fractions found was also low (Marxen and Becker, 1997; Pereira-Mouriès et al., 2002; Caiping et al., 2005; Marie et al., 2007). Yet, regarding the number of bands, these results revealed great conformity with previous studies, which state between 4 to 6 bands, depending on the mussel species (Misogianes and Chasteen, 1979; Keith et al., 1993; Moura et al., 2000). Curiously, the new data showed that the seven protein fractions present close molecular weight both on the shell matrix and extrapallial fluid samples. This finding may point out that specific proteins in the extrapallial fluid are involved on the shell biomineralization process in *A. cygnea*. Additionally, the MALDI-MS analysis revealed a large number of peptides per protein band in all extrapallial and shell fractions. There are similarities in the peptide mass from equivalent protein bands among different extrapallial samples and the same occurs in the shell samples. Furthermore, similarities were also found when comparing equivalent fractions from fluid and shell samples (Figure 4). These statements may eventually predict the presence of similar proteins in fluid and shell samples. All these aspects, in general, lead us to propose a specific functional role of fluid proteins on the shell formation.

The results of the immunogold assay in *A. cygnea* to detect organic matrix protein proved to be effective and useful. In the back-scattered electron mode, the gold particles (covalently bound to the secondary antibody) appeared as tiny bright spots. As shown in Figure 5, these tiny bright spots were mainly found in the spaces between the calcareous crystals, though some were also observed within the crystals, denoting the presence of organic matrix as a fundamental component on the biomineral phase. Actually, these spots were distributed either around the columnar structure of aragonite crystals or filling the spaces between nacreous layers of aragonite crystals. This confirms that the organic matrix may play an essential role on the mineral formation and organization in both vertical and horizontal axes in bivalve shell (Krampitz et al., 1983; Checa, 2000).

Molecular Vibrations of the Organic Matrix of the Nacreous Layer

Infrared spectroscopy is a resource for a characterization at a molecular level of the structure and bonding of surface functional groups and adsorbed species. In this study, ATR-IR spectra of aqueous organic matrix shows two molecular vibrations at 3300 cm^{-1} and 1640 cm^{-1} (Figure 6) corresponding to amide group (amide-A). The band observed at 3300 cm^{-1} is very broad because the O-H stretching band appears as a typical polymeric hydrogen bonded envelope near 3300 cm^{-1}. This means that the polymeric hydrogen bonding donors are part of the amide-A (C-H) group which displays strong and broad C-H asymmetric stretching absorptions at 3300 cm^{-1}. The molecular vibration at 1640 cm^{-1}

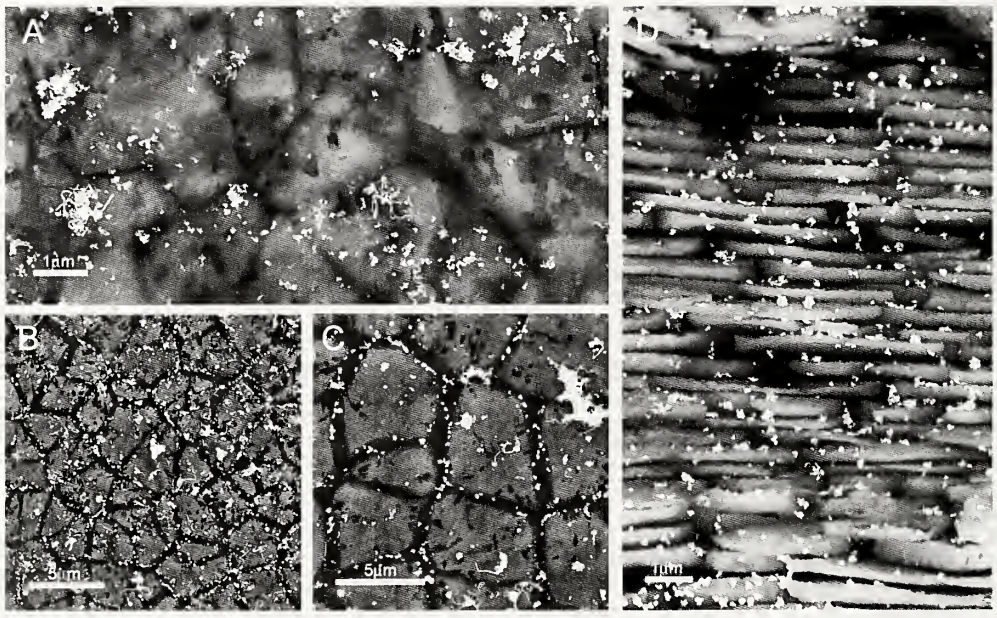

Figure 5. Immunogold assay results. Topographical view of (A) negative control, (B) and (C) test with positive results; (D) positive result in a transversal view.

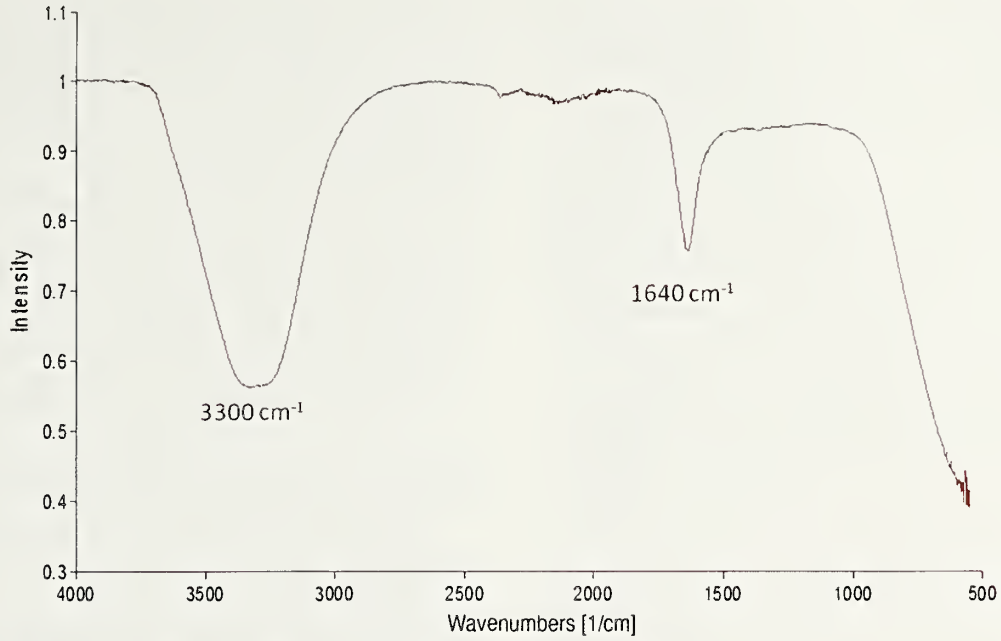

Figure 6. ATR-IR spectra of aqueous organic matrix from nacreous layer *A. cygnea*.

represents a symmetric C-O stretching. A band may be attributed to the presence of some C-O (amide 1°-band).

On the other hand, nacreous solid samples analysed (Figure 7) show a strong stretching vibration of CO_3^{2-} at 1470 cm^{-1} corresponding to ν_3 vibration mode, which is relevant for aragonite structure. The ν_2 asymmetric bending vibration at 860 cm^{-1} is also observed, which suggests a higher Ca^{2+} contribution than Sr^{2+} or Mg^{2+} in aragonite structure. The vibration at 710 cm^{-1} also corresponds to the ν_4 vibration mode (O-C-O) in plane bending of CO_3^{2-}. The vibration band at 1016 cm^{-1} corresponds to the ν_1 vibration mode of CO_3^{2-}. The shoulder at about 1600 cm^{-1} is related with amide-I functional group.

CONCLUSIONS

In general, this study provides new information on the organic shell matrix of *Anodonta cygnea* and confirmed similarities with other studied families. Despite its small

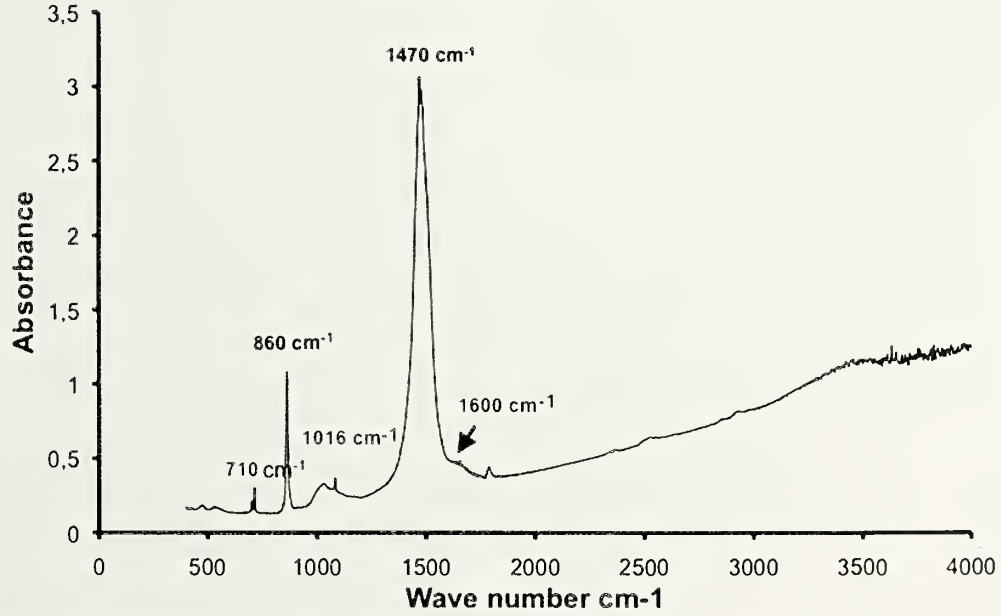

Figure 7. FT-IR spectrum of the internal nacreous layer (with aragonitic structure) collected from the freshwater mussel *Anodonta cygnea* shell.

percentage, the organic matrix, constituted mainly by proteins and sulfated GAGs, has a relevant influence on microstructure (proteins) and on the crystal nucleation (sulfated GAGs) during the formation of the calcium carbonate layers (Moura et al., 2000; Lopes-Lima et al. 2005). Additionally, it has been suggested (Tong et al., 2002) that while the intra-crystalline organic matrix, mainly composed of small negatively charged molecules, provides nucleating points and induces nucleation process (Nudelman et al., 2006), the inter-crystalline organic framework possesses a more complex organic composition and is responsible for supporting, limiting size and shape, and determining crystal growth spatial orientation.

In the present study, fluid and nacreous protein fractions, composed by similar weight molecular peptides as found by electrophoretic and MALDI-MS analysis as well as the protein matrix observed in the nacreous by SEM hystochemical techniques, seem to play mainly an inter-crystalline role in the shell biomineralization. On the other hand, the results obtained from soluble matrix and solid nacreous samples by infrared spectroscopy analyses, showing the similar occurrence of an amide group, probably points out an intra-crystalline factor contributing for the aragonite crystal formation in the nacreous layer of *A. cygnea*. In fact, according to Choi and Kim (2000), Xiao et al. (2005) and Kasat et al. (2006), the great electronegativity of oxygen allows amides to act as H-bond acceptors changing the H-bonding states of C=O groups and consequently may define secondary structure and polymer crystallinity. Possibly, the amide-I group is involved in the calcium carbonate intra-crystalline structure acting as an inductor of aragonite polymorph.

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Two new species of Velutinidae Gray, 1840 (Gastropoda) from the North Pacific with a preliminary molecular phylogeny of the family

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ABSTRACT

Two recent collections of shelled gastropods in the temperate North Pacific have identified two undescribed species of the family Velutinindae. *Marsenina zadei* new species is described from Port Townsend, Washington. *Onchidiopsis clarki* new species is described from Pribilof Island, Alaska, Bering Sea. Both species are barcoded with sequences of the mitochondrial COI and 16S genes. Additionally, a preliminary phylogeny for the Velutinidae based on these two genes is provided.

Additional Keywords: Velutinidae, Lamellariidae, *Marsenina*, *Onchidiopsis*

INTRODUCTION

Current classifications place lamellarid gastropods in the family Velutinidae Gray, 1840 (Bouchet and Rocroi, 2005). These are caenogastropods with an internal shell that have been traditionally neglected. The species-level taxonomy of this family has been in disarray. It is not the intention of this paper to review the higher taxonomic nomenclatural problems, so we follow the most recent review of species presented in Gulbin and Golikov (2001, but see 1997, 1998, 1999, 2000). Prior to that, the most comprehensive treatment of species from the North Pacific Ocean was given by Behrens (1980), which, while focusing on species known from the eastern Pacific (Alaska to Mexico), differentiated members of the genera *Lamellaria*, *Marsenina*, and *Marseniopsis*.

The main objective of this paper is to describe two new species of the genera *Marsenina* and *Onchidiopsis* (subfamily Velutininae Gray, 1842). Species of *Marsenina* are distinguished by a radula having a formula of 2.1.1.1.2 (two outer teeth are present on each side), being hermaphroditic, having a fissure or pore in the mantle exposing the shell and permitting the retraction of the mantle, and having a small, smooth foot that remains hidden under the mantle. Species of *Onchidiopsis* has the same radular formula as *Marsenina*, and are hermaphroditic, but are distinguished in that they have an internal shell fully enveloped by the mantle that is not retractile, and a long foot with a distinctive rugose or nodular edge.

In order to allocate these two species within the phylogeny of the group, preliminary molecular phylogenetic analyses (based on two mitochondrial genes) were conducted, comprising the two new species and other members of the Velutinidae for which sequences are available in GenBank.

MATERIALS AND METHODS

Collection and Preservation: Specimens were collected by scuba and trawl, respectively. All collected specimens were fixed and preserved in 95% ethanol to facilitate genetic analyses. All specimens were catalogued and deposited in the Invertebrate collection of the Natural History Museum of Los Angeles County (LACM).

Morphological Examination: Preserved specimens were dissected and the internal features were examined using a dissecting microscope. The buccal mass of one individual of each species was removed and dissolved in 10% sodium hydroxide until the radula and jaw were isolated from the surrounding tissue. The radula and jaw were then rinsed in water, dried, mounted, and sputter-coated for examination under a scanning electron microscope (SEM) Hitachi S-3000N at the LACM. The anterior end of the body, including the head and the penis, where dissected and chemically dried with hexamethyldisilazane for SEM examination.

DNA Extraction, PCR, and Analyses: DNA extraction was performed using a hot Chelex® protocol with approximately 1–3 mg of the foot cut into fine pieces. The tissue was rinsed and rehydrated using 1.0 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 20 minutes. A 10% (w/v) Chelex® 100 (100-200 mesh, sodium form, Bio-Rad) solution was prepared using TE buffer. After rehydration, the tissue mixture was then centrifuged, 975.00 µL of the supernatant was removed, and 175.00 µL of the Chelex® solution was added. Samples

were then heated in a 56°C water bath for 20 minutes, heated in a 100°C heating block for 8 minutes, and the supernatant was used for PCR. Universal16S rRNA primers (16S ar-L 5′-CGCCTGTTTATCAAAAACAT-3′, 16S br-H 5′- CCGGTCTGAACTCAGATCACGT-3′ developed by Palumbi, 1996) and universal CO1 primers (LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′, HCO2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ developed by Folmer et al., 1994) were used to amplify the regions of interest for all specimens.

The master mix was prepared using 34.75 µL H_2O, 5.00 µL Buffer B (ExACTGene, Fisher Scientific), 5.00 µL 25 mM $MgCl_2$, 1.00 µL 40mM dNTPs, 1.00 µL 10mM primer 1, 1.00 µL primer 2, 0.25 µL 5 mg/mL Taq, and 2.00 µL extracted DNA. Reaction conditions for 16S were as follows: an initial denaturation for 2 min at 94°C, 30 cycles of 1) denaturation for 30 sec at 94°C, 2) annealing for 30 sec at 50°C, and 3) elongation for 1 min at 72°C, and a final elongation for 7 min at 72°C. Reaction conditions for CO1 an initial denaturation for 3 min at 95°C, 35 cycles of 1) denaturation for 45 sec at 94°C, 2) annealing for 45 sec at 45°C, and 3) elongation for 2 min at 72°C, and a final elongation for 10 min at 72°C.

PCR produets yielding bands of appropriate size (approximately 475 bp for 16S and 700 bp for CO1) were purified using the GeneJet PCR Purification Kit (Thermo Scientifie). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 2.0 pmol/µL to send out for sequencing with the PCR products. PCR products were diluted to 7.5 and 11.5 ng/µL for 16S, and CO1, respectively. Samples were sequenced at Source Bioscience (Santa Fe Springs, CA).

For the phylogenetic analyses, sequences of the following species were obtained from GenBank: *Coriocella nigra* Blainville, 1824 (16S: AY161381, COI: AY161614), *Lamellaria* sp. 1 (16S: AY161382, COI: AY161615), *Lamellaria* sp. 2 (16S: AY161383, COI: AY161616), and *Marseniopsis mollis* (COI: GU227110). The triviid species *Trivella millardi* (Cate, 1979) (16S: AY161389, COI: AY161622) was selected as the outgroup based on recent phylogenetic analyses (Meyer, 2003). Sequences for each gene were assembled and edited using Geneious Pro 4.7.4 (Drummond et al., 2010). Geneious was also used to extract the consensus sequence between the primer regions, to construct the alignment for each gene using the default parameters and to concatenate the alignments. The sequences were trimmed after alignment. A total of 473 bp for 16S, and 614 bp for CO1 were used for the phylogenetic analyses.

The phylogenetie analyses were conducted for both genes concatenated. The Akaike information criterion (Akaike, 1974) was executed in MrModeltest (Nylander, 2004) to determine the best-fit models of evolution for each gene (GTR+I+G for COI and GTR+I for 16S). The Bayesian analysis was executed in MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001), partitioned by gene (unlinked). The Markov chain Monte Carlo analysis was run with two runs of six chains for ten million generations, with sampling every 100 generations. The default 25% burn-in was applied before constructing majority-rule consensus tree/s. The maximum likelihood analysis was conducted with the program GARLI v0.96b8 (Zwickl, 2006). Default parameters were used to run three different GARLI searches of 10 replicates each, and a total of 2,000 bootstrap replicates were performed to assess the robustness of each clade (Felsenstein, 1985).

SYSTEMATICS

Family Velutinidae Gray, 1840
Subfamily Velutininae Gray, 1840

Genus *Marsenina* Gray, 1850

Type Species: *Lamellaria prodita* Lovén, 1846 (= *Oxynoe*? *glabra* Couthouy, 1838)

Marsenina zadei new species

(Figures 1–4, 7–9)

Description: External Morphology: Mantle color makes this species difficult to find on its substrate (Figure 1). Mantle color dusky-white to tan to orange, with a sprinkling of black specks. A dark brown horseshoe mark present posterior-medially in some specimens. Mantle with a dorsal slit or fissure, which may be retracted exposing shell. Mantle with an anterior and right lateral fold creating incurrent and excurrent siphons, respectively, circulating water over gills. Surface of the mantle covered with a pattern of spots resembling atrial siphons of ascidian host. Some specimens with radiating ridges on mantle (Figure 2). A most distinctive mantle feature is a series of tubercles within the dark horseshoe (Figures 2–4).

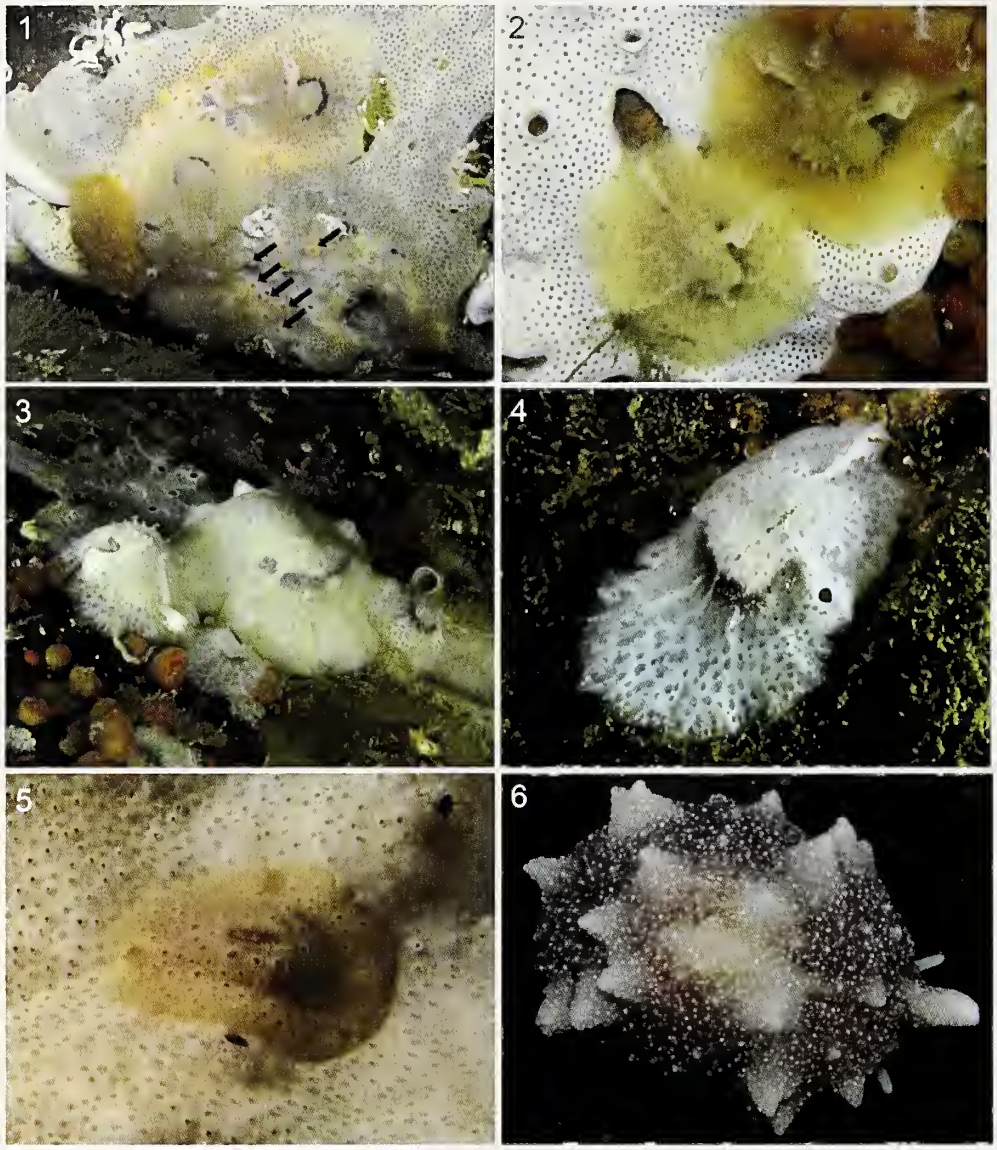
Shell: Shell oval, translucent-white, with a number of growth lines. Protoconch situated on posterior right side of shell, partially engulfed by teleoconch. Protoconch large, elongate, about 600 µm × 1 mm with 1.1 whorls (Figure 8).

Radula and Jaws: The radular formula is 98 × 2.1.1.1.2. The rachidian tooth bears 1 to 2 strong denticles to each side of the central cusp (Figure 7). The inner lateral teeth bear a single, strong denticle to each side of the central cusp. The outer lateral teeth are smooth and hamate. Masticatory border of the jaw (Figure 9) with a series of uniform denticles.

Penis: The penis (Figure 10) is flat, branching into three blunt apices distally.

Molecular Data: Sequences of this species are available in GenBank. Molecular phylogenetic analyses place this species as sister to *Onchidiopsis clarki*, but with limited support in the Bayesian analysis (Figure 14).

Biology: All specimens were collected on an encrusting compound ascidian, tentatively identified as *Tridemnum*



Figures 1–6. Living animals of *Marsenina* and *Onchidiopsis* species. **1–4.** *Marsenina zadei* new species. Port Townsend, Washington, black arrows indicate the salmon-orange egg capsules. Photos by Rick Zade. **5.** *Marsenina stearnsii* (Dall, 1871). Keysone Jetty, Whidbey Island, Washington. Photo by Jan Kocian. **6.** *Onchidiopsis clarki* new species. Bering Sea, NNE of Pribilof Island, Alaska. Photo by Roger Clark.

opacum (Ritter, 1907), at depths from 3 to 15 m. Collected with the specimens, and buried in the tunic of the ascidian, were salmon-orange egg capsules (Figure 1). Upon dissection the orange color within some of the capsules was determined to be the color of the developing larvae.

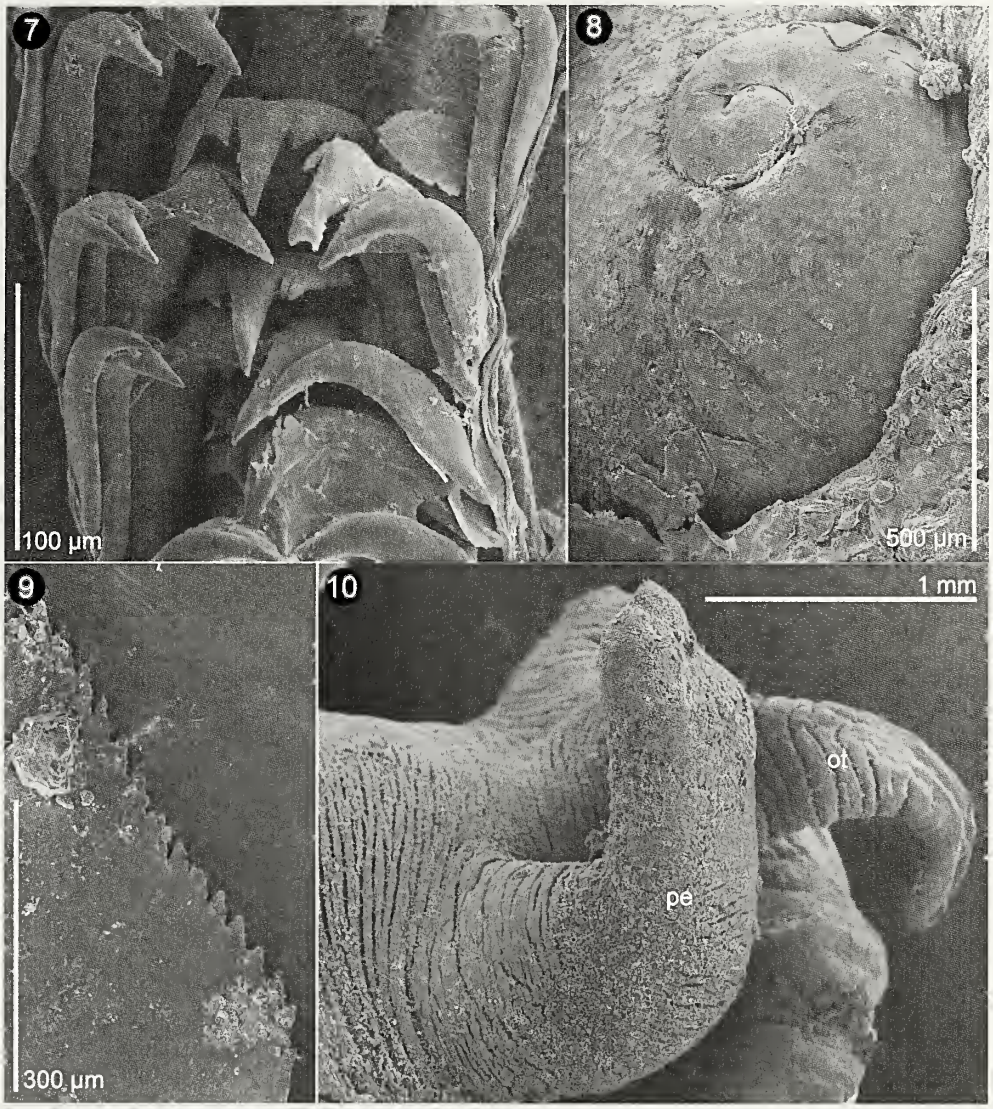
Type Material: Holotype, LACM 3280, 15 mm preserved length; Paratypes, LACM 3281, 11 specimens, 6–15 mm preserved length, all Richard Zade coll., 8 February 2010, from type locality.

Type Locality: Hudson's Point (48°6.949N, 122°44.999W), Port Townsend, Washington State, USA, 15 m depth.

Distribution: Port Townsend, Washington (present study); Ten Mile Point, Greater Victoria, British Columbia, Canada (photo by James Hester); Pigeon Point, San Mateo County, California (photos by Gary McDonald and Doug Mason); Carmel Point, Monterey County, California (photo by Gary McDonald).

Etymology: This species is named after Richard Zade, the collector of the type specimens.

Remarks: *Marsenina zadei* differs significantly from the two other described species from the North Pacific, both internally and externally. As in *M. zadei*, the mantle of *Marsenina stearnsii* resembles the encrusting tunicate, *Tridemnum opacum*, but *M. stearnsii* lacks any dark markings or black specks, and has a smooth mantle



Figures 7–10. *Marsenina zadei* new species, Port Townsend, Washington. **7.** SEM of a section of the radula, showing rachidian, lateral, and marginal teeth. **8.** SEM of protoconch. **9.** SEM of masticatory border of jaw. **10.** SEM of penis (pe) and oral tentacle (ot).

lacking tubercles (Figure 5). Dall in Orcutt (1885) and Smith (1948) state that *Marsenina stearnsii* var. *orbiculata* is not a valid taxonomic entity. Ghiselin (1964) and Behrens (1980; 1984) describe the morphology of the mantle and are the only known published photographs of living specimens of *M. stearnsii*. In *M. stearnsii*, the mantle coloration is white to creamy white with slightly elevated darker cream colored spots resembling the atrial siphons of its host ascidian (Figure 5). Numerous photos were submitted by colleagues and more found on the web that are attributable to *M. zadei*, but those had been idntified as *M. stearnsii*. Bsed on these photos, it is possible extend the geographical range of *M. zadei* south to Carmel Pt. California, December 2, 1971 (photo by G. McDonald).

Of the internal anatomy, only the radula and shell have been described for *M. stearnsii* and *M. rhombica*. Dall (1871, 1885) reported the distinguishing characteristics of the shell surface of *M. stearnsii* to be microscopic fine revolving striulae. However, such striulae were not observed on the shell a specimen of *M. stearnsii* from Marin County, California (LACM) examined for this study. The main differences between the shells of *M. stearnsii* and *M. zadei* is the protoconch morphology; in *M. stearnsii* the protoconeh is more circular in shape and has nearly 2 whorls, whereas the protoconch of *M. zadei* is more oval and has 1.1 whorls.

In most cases, as in other genera of this family, only the penis has been described for *Marsenina*. The penis of *Marsenisn zadei* is not similar to any of those species for which descriptions are available. The penis of co-occuring *Marsenina rhombica* is figured and described by Gulbin and Golikov (2000) as horn-shaped, not flattened and slightly bifurcate as described for *M. zadei* herein.

Behrens (1980) described the radulae of *M. stearnsii* and *M. rhombica*. While the number of rows of teeth in

the radula was not given for either of the north Pacific *Marsenina* species, the morphology of the teeth was found to be identical between the two and differs slightly from that of *M. zadei*. *Marsenina stearnsii* is reported to have a rachidian tooth with zero or one denticle flanking the central cusp, while the rachidian of *M. zadei* bears 1 to 2 strong denticles to each side of the central cusp. The inner and outer lateral teeth are similar in all three species.

Genus *Onchidiopsis* Bergh, 1853

Type Species: *Onchidiopsis groenlandica* Bergh, 1853

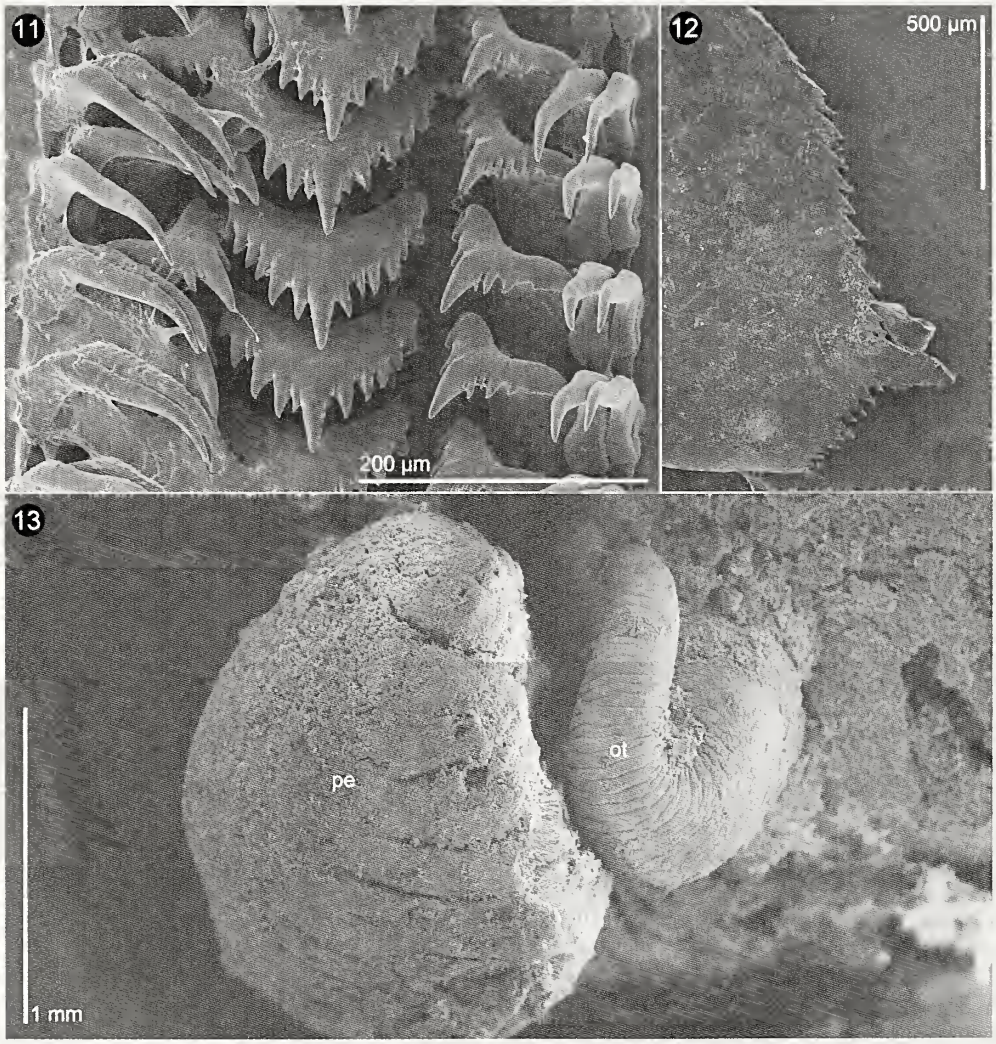
Onchidiopsis clarki new species
(Figures 6, 11–13)

Description: External morphology: Mantle completely covers shell. Mantle covered with randomly spaced, large triangular tubercles (Figure 6). An anterior fold gives origin to incurrent siphon. Oral tentacles protruding from under mantle. Oral tentacles long, slender, and tapering (Figure 13). Specimens brown with uniformly distributed white specks. Brown ground color fades to off-white on tubercles and siphon.

Shell: The shell is an un-calcified, soft plate without any recognizable characteristics in the specimens examined.

Radula and Jaws: Radular formula is 74 × 2.1.1.1.2 (Figure 11). Rachidian bears 5–7 irregularly sized denticles on each side of central cusp. Inner lateral teeth bear 3–5 dissimilarly sized denticles on each side of the central cusp. Pair of outer lateral teeth simple, hooked, lacking denticles. Masticatory margin of jaws with a series of nearly uniform denticles, posteriorly with a large denticulate flange (Figure 12).

Penis: The reproductive system is typical of members of the genus, *Onchiopsis*, from what little information we could find. The penis (Figure 13) is thickened, highly twisted, with a blunt truncated end.



Figures 11–13. *Ochidiopsis clarki* new species, Alaska, Bering Sea. **11.** SEM of a section of the radula, showing rachidian, lateral and marginal teeth. **12.** SEM of masticatory border of jaw. **13.** SEM of penis (pe) and oral tentacle (ot).

Molecular Data: Sequences of this species are available in GenBank. Molecular phylogenetic analyses place this species as sister to *Marsenina zadei*, but with limited support in the Bayesian analysis (Figure 14).

Biology: Both specimens were collected in the same trawl over a mud bottom at a depth of 87 m. There were no indications of which of the other organisms collected in the trawl might be this species prey.

Type Material: Holotype, LACM 3282, specimen 28 mm preserved length; Paratype, specimen 26 mm preserved length, LACM 3283, all trawled by the R/V Arturus, leg. Roger Clark, from type locality.

Type Locality: NNE of Pribilof Island (58°00.79N, 170°58.18W), Bering Sea, Alaska, Bering Sea, USA, 87 m depth on mud, bottom temperature 4.0°C (NMFS 88-2003-1-138).

Etymology: This species is named after Roger Clark, the collector of the type specimens.

Remarks: The genus *Onchidiopsis* is found in both the cold temperate Atlantic and Pacific Oceans. Both faunas are poorly known. Depending on which database we examined, numbers of species varied around 15 for the North Atlantic, while 10 are reported from the North Pacific (Gulbin and Golikov, 2001). Several of these are reported from throughout the Arctic Sea, reaching into both oceans.

Although Balch's (1910) original description of *Onchidiopsis corys* from Newfoundland and Labrador, North Atlantic, describes the notum as smooth on the top and sides, with wrinkles and folds elsewhere, photos on the web (http://eol.org/pages/72611/overview and http://eol.org/pages/593815/overview) of recent specimens "thought to be" *O. corys* bear some external similarity with *O. clarki*, having triangular tubercles on the mantle. These specimens have lighter color and their mantle surface is knobby and granular between the tubercles. Balch's description of the internal anatomy is vague and could apply to any species in the genus.

The most obvious Circumboreal species to be considered here are *Onchidiopsis glacialis* (Sars, 1850) and *Onchidiopsis groenlandica* Bergh, 1853. These species seem to have been maintained as separate in the literature (Bergh, 1886; MacGinitie, 1959; Gulbin and Golikov, 2001) even though there appears to be adequate arguments to synonymize the two (Balch, 1910; Thorson, 1944; Macpherson, 1971). Where the mantles of these two very similar species are discussed, descriptions vary from rugose and wrinkled (Gulbin and Golikov, 2001) to convoluted (brain-like) (Macpherson, 1971). We have found no mention of specimens with triangular tubercles.

Nowhere in the literature can we find a North Pacific species with large triangular tubercles seen in *O. clarki*.

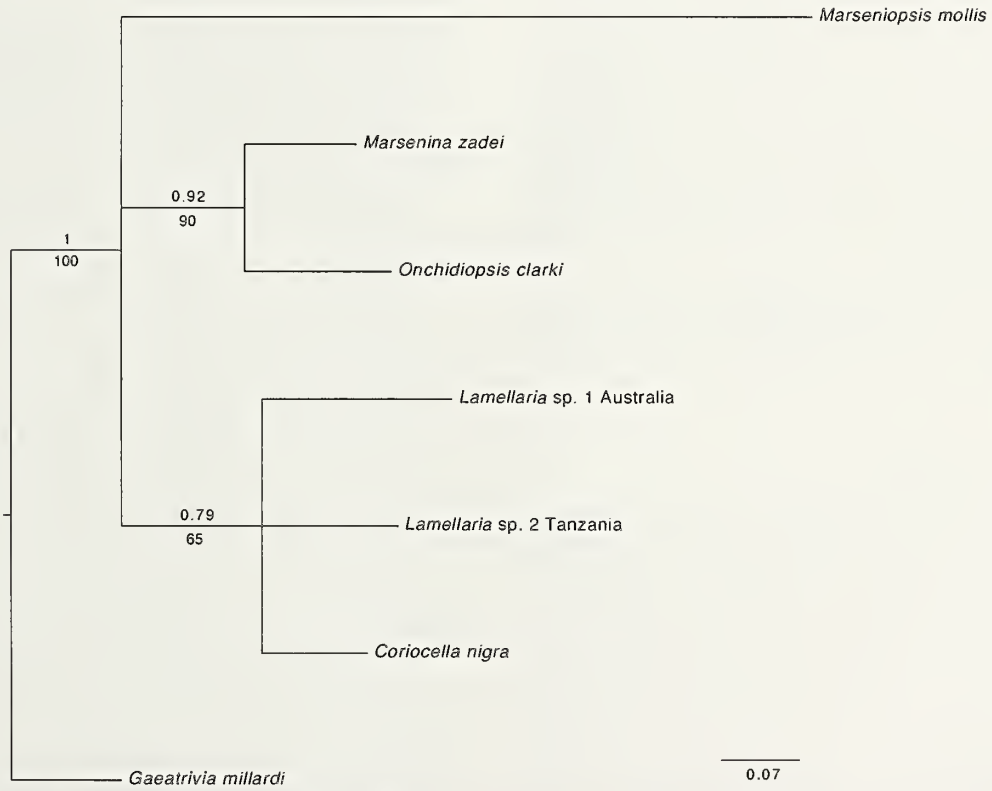


Figure 14. Bayesian consensus tree of the concatenated analysis including posterior probabilities and bootstrap values from the maximum likelihood analysis.

All of the species figured in Gulbin and Golikov (2001) appear to have smooth or slightly granular mantle surfaces, lacking tubercles.

We were unable to find any published description of the internal reproductive system, only that of the penis, which in the genus seems to be diagnostic. Of those species described by Gulbin and Golikov (2001), none are comparable to the penis of *O. clarki* described here as thickened, highly twisted, with a blunt truncated end. In *O. groenlandica*, they described the penis as having an ancillary appendage hanging over the penis. In *O. variegata*, they described the penis as long, cylindrical, its distal part thickened and curved, terminating in a swelling with a thin fold. In *O. zuclusi*, they describe the penis as highly characteristic with a large divided lobe. On the side it bears an extending papilla, rimmed with a thin fold. All members of the subgenus *Rostrooncli-idiopsis* have a long tapering, hook shaped penis with a crest at the bend, while members of the genus *Bulloonchidiopsis* have a flattened, hammer-shaped penis, the distal end of which is recurved upward.

DISCUSSION

In this paper we include the first, albeit very preliminary, molecular phylogeny of the Velutinidae, based on COI and 16S sequence data. The resulting tree lacks support for most branches, suggesting that the two genes sequenced are not adequate to recover the phylogeny of the Velutinidae. However, the general structure of the tree appears to partially support the classification scheme proposed by Bouchet and Rocroi (2005). The genera *Lamellaria* and *Coriocella* (subfamily Lamellariinae) are placed in the same clade. However, the other subfamily recognized by Bouchet and Rocroi (2005), Velutininae, is paraphyletic in the present analysis, as *Marseniopsis* does not cluster with *Marsenina* and *Oncluidiopsis*, which form a monophyletic group well supported in the maximum likelihood analysis.

In order to reconstruct the phylogeny of this group it will be necessary to sequence additional genes, including nuclear markers and substantially expand the taxonomic coverage.

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The authors would like to thank the two gentlemen who made us aware of these two interesting species. Richard Zade, avid diver and master underwater photographer from Spanaway, Washington, somehow noticed this very cryptic species hiding on its host tunicate. Thanks also to Roger Clark, contract biologist to the National Marine Fisheries Service, who was responsible for invertebrate identification from trawl samples aboard the R/V Arcturus. Ironically, he is specialist in crabs! The authors would also like to thank James Hester, Gary McDonald, and Doug Mason for providing photographic evidence of *Marsenina zadei* occurring in Canada and California, and Jan Kocian for the photo of *M. stearnsii* from Whidbey Is. Washington. Lindsey Groves (LACM) curated the specimens studied and provided us with access to the collection. The SEM work was conducted at the LACM SEM facility sponsored by the NSF (MRI grant DBI-0216506) with the assistance of Giar-Ann Kung.

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A new genus of Buccinoidea (Gastropoda) from Paleocene deposits in eastern Hokkaido, Japan

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ABSTRACT

A new genus and a new species of the gastropod superfamily Buccinoidea, *Urahorosphaera kanekoi*, is described from the Paleocene Katsuhira Formation in eastern Hokkaido, Japan. This species comprises the first Paleocene record of buccinoid gastropods in Japan and originates from relatively deep-water deposits, which makes it very unique among all Paleocene buccinoidean gastropods in the North Pacific. This occurrence may suggest a trace of bucciniform gastropods diversification similar to the pattern found for the Southern Hemisphere.

Additional Keywords: New species, *Brachysphingus*, *Austrosphaera*, *Seymourosphaera*

INTRODUCTION

Late Cretaceous and Paleocene buccinoidean gastropods of uncertain affinity are known from the North Pacific, Antarctica, and South America. The genus *Brachysphingus* Gabb, 1869 is known from the Paleocene (Danian–Thanetian) of Kamchatka (Gladenkov et al., 1997) and California (Squires, 1997), genus *Austrosphaera* Camacho, 1949 from the Late Cretaceous and Paleocene strata of Tierra del Fuego, and genus *Seymourosphaera* Oleinik and Zinsmeister, 1996 from the Paleocene (Danian) of Seymour Island, Antarctica (Oleinik and Zinsmeister, 1996; Stilwell et al., 2004). The last two genera have once been tentatively included in the subfamily Pseudolivinae Cossmann, 1901 (Oleinik and Zinsmeister, 1996). Vermeij (1998) excluded them from Pseudolivinae based on the lack of a pseudolivid groove and a labral tooth. Based on their occurrence in the Southern Hemisphere, the genera *Austrosphaera* and *Seymourosphaera* most probably belong to the austral family Buccinulidae Finlay, 1928, rather than to the North Pacific family Buccinidae Rafinesque, 1815. All these buccinoid genera share a semi-ovate outline of the shell, predominantly smooth surface, low to moderately elevated spire, short siphonal canal and a poorly developed fasciole.

No Paleocene buccinoidean gastropods of similar affinities were previously reported from Japan. Two specimens of smooth-surfaced, subovate buccinids have recently been collected from the Paleocene part of the Katsuhira Formation (Amano and Jenkins, 2014) in eastern Hokkaido. We herein propose a new genus and a new species for these unusual buccinids.

MATERIALS AND METHODS

Two buccinoidean specimens were collected from two separate carbonate concretions, 20 to 40 cm in diameter, found as floats. Concretions were originally embedded within the mudstone of the Katsuhira Formation, eastern Hokkaido (Figure 1; Loc. 1 and 2). Although most concretions at these localities occurred as floats, they must have been derived from localities nearby. For example, some autochthonous concretions yielding fossils were cropped out 50 m upstream of Loc. 1.

Many calcareous concretions with plant debris are found in the upper part of this formation near the type locality. As discussed by Amano and Jenkins (2014), the age of the upper part of this formation has been assigned to the Paleocene, based on planktonic foraminifers. The age of the upper part of the Katsuhira Formation was assigned to early Selandian, based on planktonic foraminifers and calcareous nannofossils (Kaiho, 1984).

One well-preserved gastropod specimen was obtained from a float calcareous concretion (30 cm in diameter) at 900m upstream of the small river, 1.5 km south to Ponkatsuhira-zawa (Loc. 1). Another, rather poorly preserved specimen was collected from a float calcareous coneretion (about 30cm in size) at approximately 1 km upstream of Ponsetarai River (Loc. 2). Multiple fragments of plant material, protobranch bivalves as *Acila*, *Leionucula*, and malletiids, aporrhaid gastropod *Kangilioptera inouei* Amano and Jenkins, 2014 and scleractinian corals are associated with the buccinoidean specimens in both localities. One of the corals was found near the aperture of the poorly preserved specimen at Loc. 2. From other localities of the Katsuhira Formation, deep-water arcoid

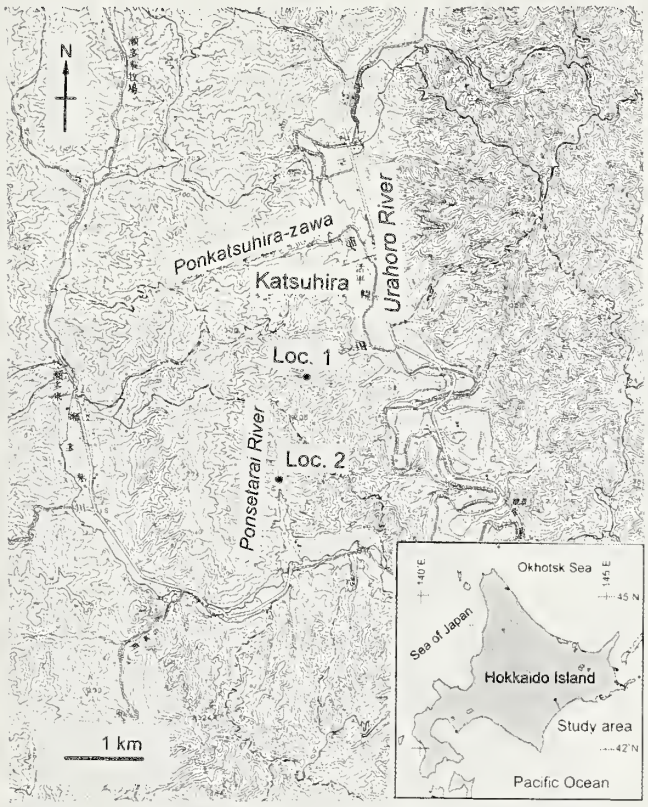


Figure 1. Locality map of *Urahorosphaera kanekoi* new genus and new species (base map is from "Tokomuro", scale 1: 50,000 topographical map published by the Geospacial Information Authority of Japan).

Bentharca was also found. The paleoenvironments of the Katsuhira Formation has not been studied in any detail. Fossils assemblage is indicative of deep-water accumulation.

Abbreviations used are: JUE: Joetsu University of Education, Joetsu, Niigata Prefecture; FAU: Florida Atlantic University, Boca Raton, Florida; PRI: Paleontological Research Institution, Ithaca, New York.

SYSTEMATIC PALEONTOLOGY

Class Gastropoda Cuvier, 1797
Order Neogastropoda Wenz, 1938
Superfamily Buccinoidea Rafinesque, 1815
Family (?)Buceinidae Rafinesque, 1815

Remarks: The Buccinidae is one of the most diverse families of neogastropods. Members of the family are distributed from the Equator to the poles, and inhabit exclusively marine environments. The current understanding is to place the northern hemisphere genera in the family Buecinidae, and the southern hemisphere genera in the family Buccinulidae (Bouchet and Warén, 1986; Schnetler, 1997; Harasewych and Kantor, 1999, 2004; Squires and Saul, 2000; Kantor and Harasewych, 2013). Problems of Buccinidae origin, appearance in the fossil record, and relationship to families Fasciolariidae, Nassariidae, and Melogenidae, have been debated in the literature for some time (Ponder, 1974; Tracey et al., 1993; Bandel, 1993; Kantor, 1996; Ponder and Lindberg, 1997). The phylogenetic relationships of the recent Buccinidae remain unclear. There is no agreement on the exact limits of the family, as well as relationships among it's over than 200 genera and subgenera (Harasewych, 1998). The molecular phylogenetic data for buceinids remain insufficient. Whatever phylogenetic data exist mostly point on the paraphyly for the Buccinidae, but also suggest the limited resolving power of current molecular phylogenetic analyses (Hayashi, 2005; Oliverio and Modica, 2010; Kantor et al., 2012).

The fossil record indicates the diversification in bucciniform gastropods during the Late Cretaceous and early Cenozoic. A leading tendency in multiple publications is to place these genera in the present-day families Buccinidae or Nassariidae. Allmon (1990) had commented that placing late Mesozoic and early Cenozoic bucciniform gastropods into a few traditionally recognized living families obscures the phylogeny and leads to underestimation of family-level diversity during this time interval. Squires (1997) had commented that in all probability, Late Cretaceous and early Cenozoic bucciniform gastropods most probably belong to several new undescribed families that are waiting to be properly erected.

It is most likely that the new genus described in this manuscript belongs to a separate, undescribed family of bucciniform gastropods. Limitations in quantity and quality of our material, however, prevent us from designating a new family with confidence at this point.

Urahorosphaera new genus

Type Species: *Urahorosphaera kanekoi* new species, Paleocene (early Selandian), upper part of the Katsuhira Formation, Urahoro Town, eastern Hokkaido, Japan.

Diagnosis: Shell subovate, inflated; spire low; protoconch bulbous; surface smooth and glossy, except for weak axial riblets near aperture; thick peristomate outer lip; siphonal canal short with weakly developed siphonal notch.

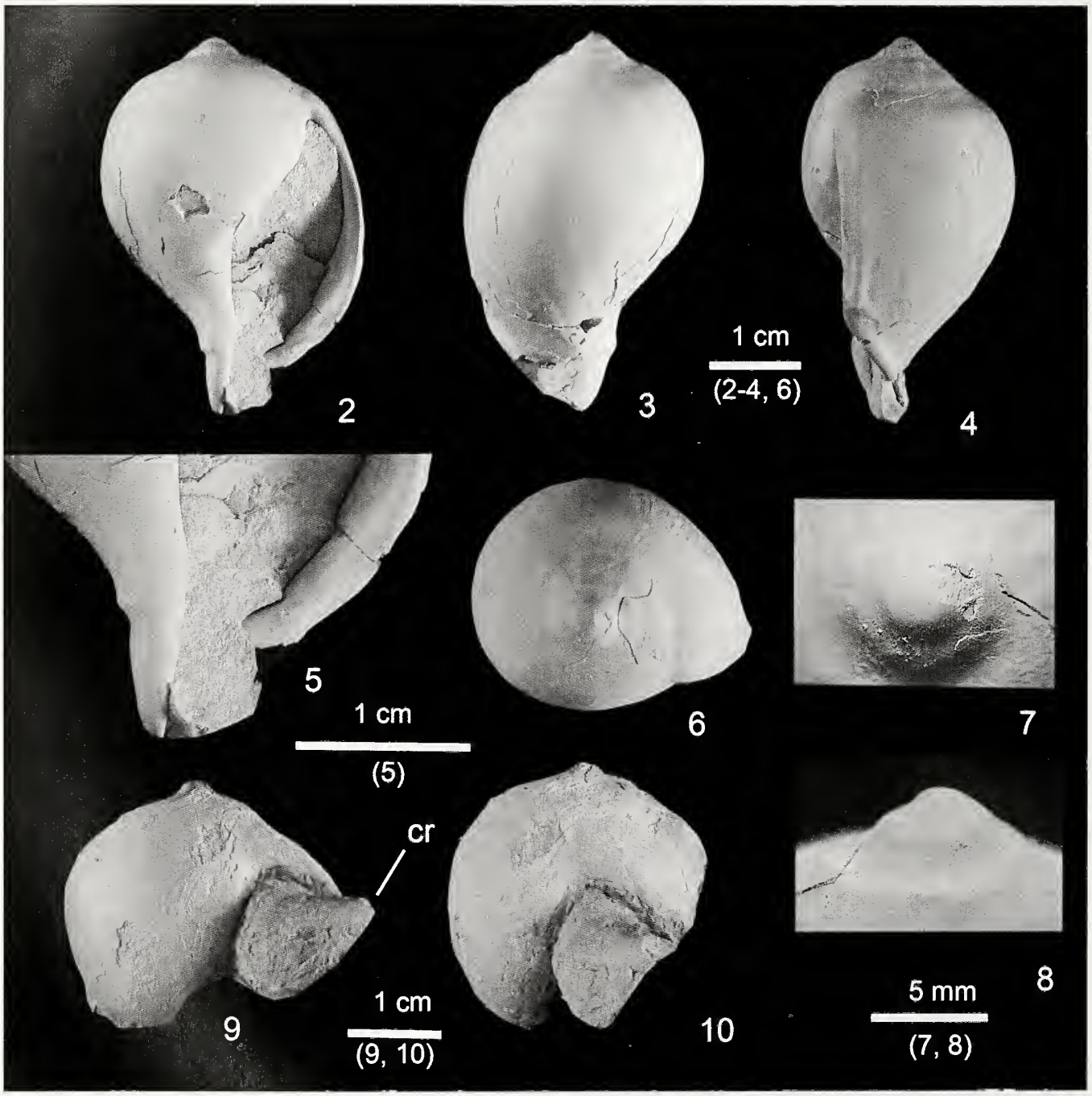
Description: Shell medium-sized, thick, with glossy surface, subovate. Last whorl large and globose; spire very low; protoconch smooth and bulbous. Surface of last whorl sculptured by thin and low axial riblets; shallow, but distinct subsutural groove; aperture pear-shaped; outer lip thick, forming peristome; thin callus covering body whorl, spire and protoconch; weakly developed siphonal notch and parietal canal; anterior end of columella abruptly tapered.

Remarks: Species in *Urahorosphaera* have broad thin callus covering whole surface. Such character is usually seen in members of the Olivoidea. However, Landau and Marquet (1999) described buccinoidean gastropod, *Cyllene* (*Cyllenina*) *lucenensis* from the Pliocene deposit

in Spain which has a callus covering teleoconch whorls. This genus is also considered to be an aberrant grouping within Buccinoidea.

Urahorosphaera resembles *Brachysphingus* Gabb, 1869, from the Paleocene to Eocene formations of California in having a subovate shell form, low spire, and short siphonal canal. However, *Urahorosphaera* differs from *Brachysphingus* by having overall larger size, a smooth glossy shell surface, and a thick outer lip and lacking of spiral cords on the base.

The austral genus *Austrosphaera* Camacho, 1949 (in Furque and Camacho, 1949) from the Late Cretaceous to Paleocene in Argentina is another similar to the *Urahorosphaera* in having overall subovate shell and low spire. *Urahorosphaera* differs from *Austrosphaera* by having a glossy shell surface, wider callus, thick outer



Figures 2–10. *Urahorosphaera kanekoi* new genus and new species. **2–8.** Holotype, JUE no. 15924; 2, apertural view; 3, adapertural view; 4, side view; 5, enlargement of base; 6, apical view; 7, apical view of protoconch; 8, side view of protoconch. **9–10.** Paratype, JUE no. 15925; 9, apertural view, cr, coral; 10, oblique view of aperture.

lip, and by lacking multiple columellar plications toward the anterior end of the columella.

The Antarctic Paleogene genus *Seymourosphaera* Oleinik and Zinsmeister, 1996 differs from *Urahorosphaera* by having multiple fine spiral threads and poorly developed siphonal canal, and by lacking a siphonal notch.

Urahorosphaera differs from *Pangoa* Marwick, 1931, from the Miocene (Lillburnian) of New Zealand by having a more compressed shell with lower spire, shorter siphonal canal, broader callus, and glossy shell surface.

Urahorosphaera differs from *Sycostoma* Cox, 1931 from the Eocene of Europe and North America by having a more rounded semi-ovate shell, lower spire, wider callus, shorter siphonal and weakly developed parietal canals, and larger bulbiform protoconch.

The genus *Liochlamys* Dall, 1889 (family Fasciolariidae) from the Neogene of the southeastern United States, although has a glossy shell surface, overall globose shape of the shell, and bulbiform protoconch, differs from the *Urahorosphaera* by having two to three columellar folds, or plications, wider and more elongated siphonal canal, higher spire, and presence of apertural ribs in the interior of the aperture.

Etymology: The new genus is named after the town of Urahoro, site of the type locality in Hokkaido.

Age and Occurrence: Paleoeene Katsuhira Formation in Urahoro, eastern Hokkaido.

***Urahorosphaera kanekoi* new species**
Japanese name: Urahoro-migaki-bora
(Figures 2–10)

Diagnosis: Same as that of the new genus.

Description: Shell medium-sized, attaining 43.0 mm in height, thick, polished, subovate with four whorls. Last whorl large and globose; spire very low, covered by thin glaze, comprising approximately 1/6 of the total shell height; protoconch smooth, bulbous, low-domed, consisting of 1.5 whorls. Surface of last whorl sculptured by twelve thin and low axial riblets near aperture; riblets becoming obsolete anteriorly. Subsutural groove very shallow, but distinct. Aperture pear-shaped; outer lip thick, forming distinct peristome. Outer lip forming blunt angle near boundary between base and posterior end; columella concave and smooth; inner lip broadly covered by thin calcareous callus; callus extending over front of shell and over parietal sinus, covering suture and protoconch; siphonal canal short and slightly oblique with weakly developed siphonal notch; parietal canal weakly developed; anterior portion of columella tapering abruptly.

Type Material: Holotype, JUE no. 15924 (shell height, 43.0 mm; diameter, 29.5 mm); Paratype, JUE no. 15925 (diameter, 28.2 mm).

Type Locality: 900 m upstream of the small river, 1.5 km south to Ponkatsuhira-zawa, Urahoro, eastern Hokkaido.

Remarks: *Urahorosphaera kanekoi* new species shares a subovate shell with low spire and generally smooth surface with *Brachysphingus mammilatus* Clark and Woodford, 1927 (especially paratype UCMP 31235; see Squires, 1997, figs. 5–10, 11) from the upper Paleocene and lower Eocene in California, and with *Brachysphingus gibbosus* Nelson, 1925 from the early Paleocene of Kamchatka (Figures 11–12). However, *Urahorosphaera kanekoi* new species differs from these species by the presence of a thick outer lip, suddenly tapering anterior end and lack of spiral cords on the basal part. *Urahorosphaera kanekoi* is similar to *Seymourosphaera bulloides* Oleinik and Zinsmeister, 1996 (Figures 13–14,) in general shape and smooth surface, but differs by a higher spire, thicker peristome, suddenly tapering anterior end, and broader callus.

Figures 11–14. Paleocene smooth-surfaced subovate gastropods. **11, 12.** *Brachysphingus gibbosus* Nelson, 1925. PRI 49420. 11, aperural view; 12, abapertural view. Paleocene, Danian, Getkilninskaya Formation, Northwestern Kamchatka. **13, 14.** *Seymourosphaera bulloides* Oleinik and Zinsmeister, 1996. FAU 18384-11. 13, apertural view; 14, abapertural view. Paleocene, Danian, Sobral Formation, Seymour Island, Antarctica.

Etymology: The new species is named for Mr. Atsushi Kaneko who collected the holotype.

Distribution: Known from the type locality and 1 km upstream of Ponsetarai River, Katsuhira Formation, Urahoro, eastern Hokkaido.

DISCUSSION

Most of the North Pacific buccinids, other than the Paleocene through middle Eocene genera *Siphonalia* and *Snatolia* (Family Siphonaliidae, according to Goryachev, 1987, Oleinik, 1988, and Gladenkov et al., 1988), appeared in the late Eocene and their diversity gradually increased from the late Eocene to recent following the general trend of climatic cooling in the region (Titova, 1994; Gladenkov et al., 1997). The Late Cretaceous and Paleocene record of the North Pacific Buccinidae remains rather poorly known. Apart from apparently buccinoidean, highly sculptured gastropods, such as genera *Ornopsis* Wade, 1916 and *Deussenia* Stephenson, 1941 from the Late Cretaceous and Paleocene of California and northern Mexico, the only early Paleocene buccinoidean gastropod, morphologically similar to *Urahorosphaera*, in the northern circum-Pacific, is the genus *Brachysphingus*. Three species of *Brachysphigus* (*B. gibbosus* Nelson, 1925, *B. sinuatus* Gabb, 1869, and *B. mammilatus* Clark and Woodford, 1927) are known from the uppermost Cretaceous (?)–lower Paleocene strata of California and Baja California (Squires, 1997). Two species (*B. sinuatus* and *B. gibbosus*) are known from the Paleocene Getkilninskaya Formation of northwestern Kamchatka (Gladenkov et al., 1997), which comprises the northernmost record of this genus in the northern circum-Pacific. The distribution of the genus *Brachysphingus* in the Paleocene of the northern circum-Pacific follows the general pattern outlined by Oleinik (2001). At that time, marine isotopic records do not indicate a significant departure in temperature values from the Late Cretaceous and do not indicate a significant thermocline of depth (Bralower et al., 2002; Dutton et al., 2005), with some indications of warmer temperatures (Adatte et al., 2002) during the early Paleocene. These paleoceanographic conditions would promote the dispersal of molluscan faunas across the northern rim of the Pacific Ocean which explains similarity of the eastern North Pacific and northwestern Pacific Paleocene molluscan faunas. Paleocene molluscan faunas from Japan remained virtually unknown until recently (Amano and Jenkins, 2014). The new genus *Urahorosphaera* is morphologically distinct from the *Brachysphingus*, showing more similarity with the austral genera *Austrosphaera* and *Seymourosphaera* that diversified in the southern hemisphere. Discovery of the *Urahorosphaera* in the lowermost upper Paleocene of Hokkaido may suggest a trace of similar early Paleocene diversification in the North Pacific. The genus *Brachysphingus* in the Paleocene of the North Pacific is also known from a shallow-marine deposits represented by lithic sandstone and associated with shallow-water bivalves. On the other hand, the lithology and assemblage of the Katsuhira Formation, from which *Urahorosphaera* was found, is indicative of relatively deep-water deposits, which makes it unique among other North Pacific Paleocene localities. Somewhat similar deep-water assemblages are found in the lower portion of the Getkilninskaya Formation of northwestern Kamchatka, but those do not contain *Brachysphingus*, *Urahorosphaera*, or any apporhaid gastropods.

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First report of *Cryptoplax propior* Is. and Iw. Taki, 1930 (Polyplacophora: Cryptoplacidae) in Korea

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ABSTRACT

We report the first occurrence of the chiton *Cryptoplax propior* Is. and Iw. Taki, 1930, a species of Polyplacophora newly reported for the Korean molluscan fauna. Two specimens of *C. propior* were obtained during faunal surveys from two localities on both the north and south coasts of Jeju Island, off the southern coast of Korea. Although reference has been made to *C. propior* in several faunal and taxonomic studies, little is known about the ecology of this species, and only a few specimens have been collected since it was first described in 1930. Scanning Electron Microscopy (SEM) revealed the characteristics of *C. propior*: the granular rows on the median and tail valves, and the short, thick blunt spicules on the perinotum. Due to the present study, the distribution range of *C. propior* now extends from south-central Japan to Jeju Island, southwestern Korea.

Additional Keywords: Jeju Island

INTRODUCTION

Located in the northern East China Sea, Jeju Island has a warm humid temperate climate with an average yearly temperature of 16°C (Korean Meteorological Administration, 2013). The ocean around the island is a complex region, where three major water masses from the north and northwest are mixed. The warm Tsushima current washes the southern coastal area of the island, giving this area somewhat warmer winter sea surface temperatures than the rest of the country, ranging from 14-16 °C (Limpanont et al., 2010, 2011). During the summer, the sea surface temperature in Jeju often reaches a maximum temperature of 28 °C (Global Sea Temperature, 2013). Since Jeju Island is part of the Warm Temperate Northwest Pacific Province (Spalding et al., 2007), there is a strong affinity with the fauna of southern Japan and eastern China, and also northeast Taiwan (Noseworthy et al., 2007). Furthermore, many warmer water species of the Indo-West Pacific region also occur in Jeju Island. For the past 12 years surveys have been conducted to enumerate the mollusk fauna, with special attention to its biodiversity, biogeography, and ecology. According to Noseworthy et al. (2007), there are 1,072 mollusk species in Jeju Island, and 755 species are exclusively distributed in marine environments. Due to the extensive surveys, several species newly reported for the island fauna have been reported (Noseworthy and Choi, 2010; Noseworthy et al., 2012).

Currently, 12 species of Polyplacophora are known to occur in Jeju Island (Min et al., 2004; Noseworthy et al., 2007), and several other specimens which may represent new species records have been obtained. *Cryptoplax* occurs mainly in the warm Indo-West Pacific region as well as in cooler regions further south, such as South Africa, Tasmania, and Western Australia (Malacos.com, 2006).

In Japan four species of *Cryptoplax* have been reported (Saito, 2000), but only *C. japonica* Pilsbry, 1895 has been identified in Korea. This species ranges from Sakhalin Island southward to the southern Korean peninsula and Jeju Island. Here we report the occurrence of *Cryptoplax*

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propior Is. and Iw. Taki, 1930, a species newly reported for the Korean molluscan fauna.

MATERIALS AND METHODS

Two chiton specimens were obtained by SCUBA diving in the autumn of 2012 at Munseom, a small island about one kilometer south of Seogwipo harbor on the south coast of Jeju Island, and at Bukcheon-ri on the north coast (Figure 1). Both specimens were obtained at a depth of 5 m. These specimens were identified as belonging to Cryptoplacidae but did not resemble any members of this family found in the Korean fauna literature (for instance, Kwon et al., 1993, 2001; Min et al, 2004). An examination of the Polyplacophora in Saito (2000) and other Japanese literature revealed that it was a specimen of *Cryptoplax propior* Is and Iw. Taki, 1930. The species has not been previously reported from the Korean Peninsula.

The specimens were measured, examined with a stereo microscope, then fixed in 70% ethanol. They were compared with the original description of *C. propior* and with specimens of *C. japonica*, which most closely resembles *C. propior*. Valves and girdle were dissected from one of the specimens for further study, and a scanning electron microscope (JSM-6700F, JEOL Korea Ltd.) was used for the examination of each valve and the perinotum spicules. The material is stored at the School of Marine Biomedical Science, Jeju National University (#L145S1005).

As the Jeju Island material is separated from the original material in both space and time, it was deemed appropriate to redescribe the valves and girdle, the most distinctive features of this species. An examination of the morphology of the radula would have been useful but such a study could not be conducted because the specimens had been dried before being placed in ethanol. This created difficulties in extracting the radula and rendered it unusable. Radula studies can be done if more material becomes available in the future. The taxonomy used follows that of Sirenko (2006), and the descriptive nomenclature is based on that of Schwabe (2010).

An appendix to this work, by the same authors, **Appendix 1. A Catalogue of *Cryptoplax* Species,** is posted online at http://nautilus.shellmuseum.org.

SYSTEMATICS

Order Chitonida Thiele, 1909
Superfamily Cryptoplacoidea H. and A. Adams, 1858
Family Cryptoplacidae H. and A. Adams, 1858

Genus *Cryptoplax* Blainville, 1818

Type Species: *Cryptoplax larvaeformis* (Burrow, 1815), by subsequent designation.

Diagnosis: Mainly moderately-large to large vermiform chitons with reduced tegmentum and wide, rather fleshy girdle covered with spicules of varying shape and size.

Cryptoplax propior Is. and Iw. Taki, 1930

(Figures 1–19)

Type Locality: "Prov. Shima" (Shima Peninsula, now part of Mie Prefecture, southeastern Japan).

Material Examined: Jeju Island, Seogwipo-shi, Munseom (one specimen); Jeju Island, Bukcheon-ri (one specimen.)

Dimensions: Length, 22 mm, width, 7 mm (extended) (Munseom); Length, 8 mm, width, 4 mm (curled) (Bukcheon-ri.)

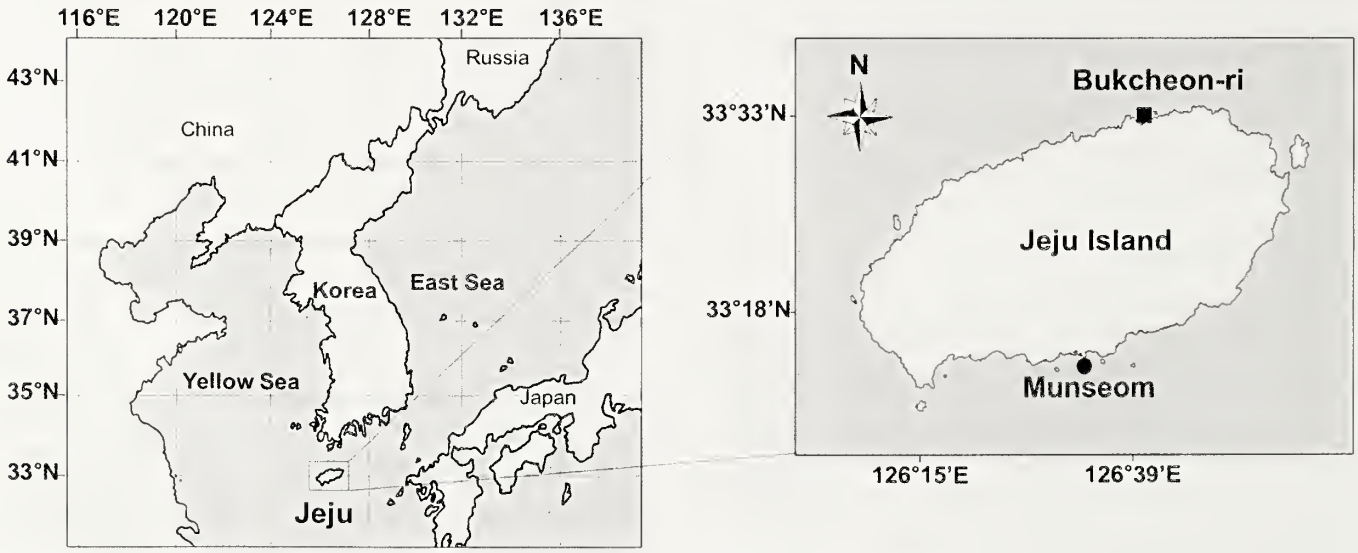
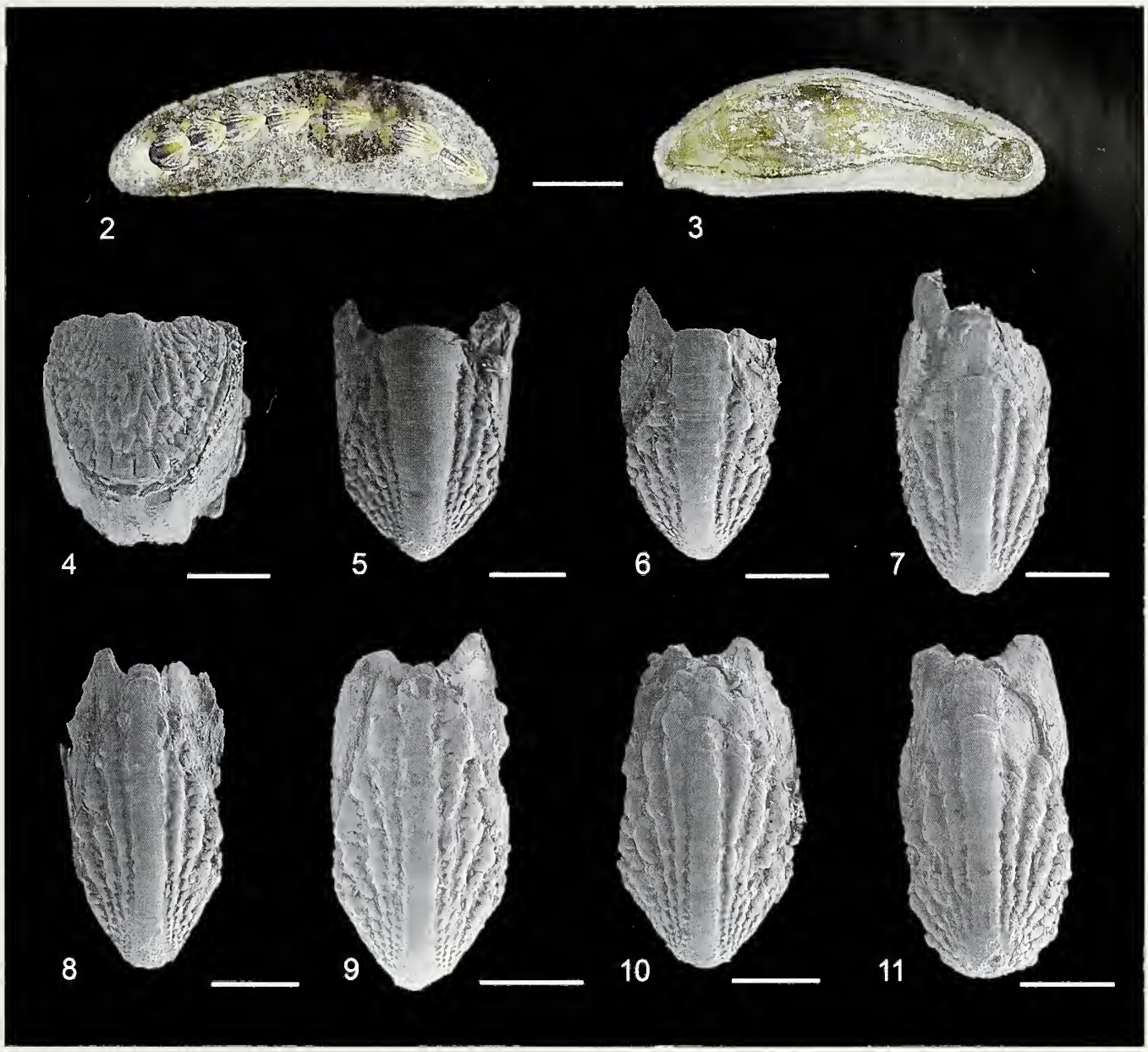


Figure 1. Sampling sites of specimens of *Cryptoplax propior*: ■: Bukcheon-ri; ●: Munseom



Figures 2–11. *Cryptoplax propior.* **2, 3.** Munseom, Jeju Island. **2.** Dorsal view. **3.** Ventral view. Scale bar = 1 cm. **4–11.** SEM view of ultrastructure of individual valves. **4.** Head valve. **5.** Second valve. **6-10.** Median valves. **11.** Tail valve. Scale bar = 1 cm.

Description: Tegmentum strongly reduced. Anterior four valves slightly overlapping; posterior four valves more widely separated (Figure 2). Color golden-brown; grooves between granulose rows somewhat lighter. Head valve rounded with moderately straight posterior margin, and possessing several indistinct granulated radial ridges and growth lines (Figure 4). Second valve round with wide, smooth jugum, tapering posteriorly, with low, longitudinal, lightly-beaded ridges. Apophyses moderately long, well-developed, with rather wide, shallow jugal lamina (Figure 5). Third to seventh valves (Figures 6–10) with about eight well-defined, slightly radiating, rather granulose rows on pleurolateral areas; jugum distinct, nearly parallel-sided. Valves narrow with slightly-beaked posterior ends; apophyses and jugal laminae similar to those of second valve but with narrower laminae. Tail valve (Figure 11) possesses posteriorly-terminal muero directed backwards, apex overhanging terminal margin; antemueronal area exhibiting well-developed jugum. Radiating granular rows, apophyses, and jugal laminae similar to those of other valves. Length, height, and jugum length of valves provided in Table 1. Perinotum with dense, short, rather blunt spicules of unequal size, light purplish-brown mottled with grayish-tan, with light tan base. Girdle fringe grayish-white with irregular, indistinct reddish-brown areas, possessing rather short, rounded spicules (Figure 2). Foot grayish-brown with posterior area somewhat pointed (Figure 3), hyponotum light tan.

Table 1. *Cryptoplax propior.* Length, height, and jugum length of individual valves, from anterior to posterior (Munseom specimen). Measurements in mm.

Valve	Shell length	Shell height	Jugum length
1	2.179	2.003	–
2	2.325	2.981	0.873
3	1.916	2.768	0.547
4	1.755	3.289	0.423
5	1.843	3.445	0.395
6	1.766	3.263	0.314
7	1.864	3.263	0.324
8	1.710	3.248	0.410

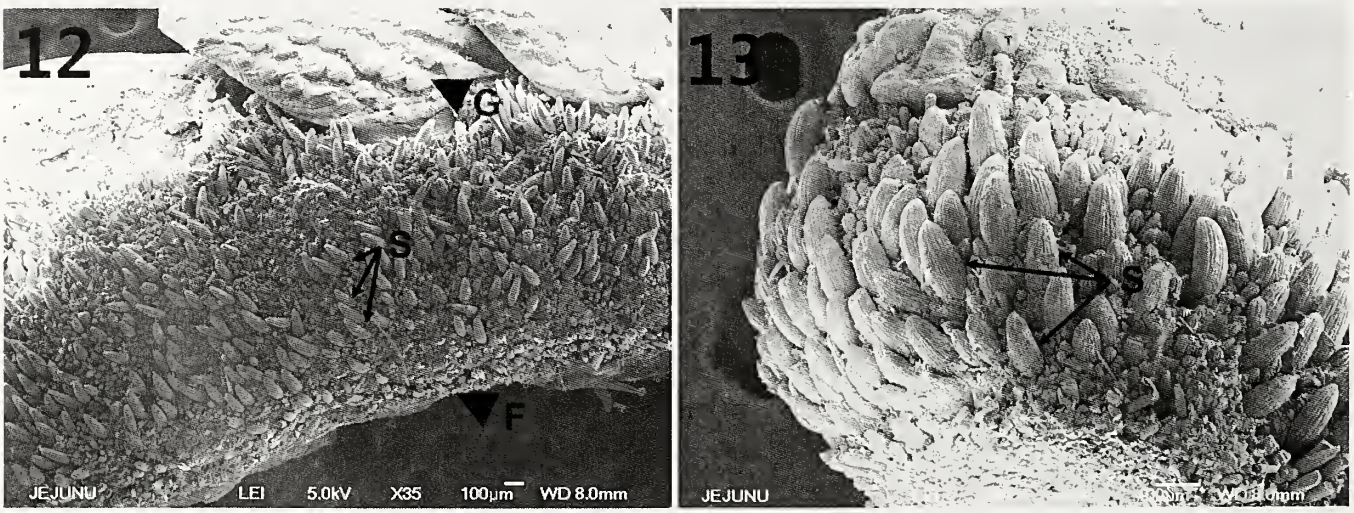
Remarks: The shape of the second, smaller specimen is more uniform, with parallel sides. The valves are similar in morphology to the dissected specimen, but are somewhat closer, suggesting a subadult specimen. However, there is no clear division between the head valve and the perinotum, the head valve appearing to blend into the orange-brown perinotum, which has dense, short, blunt spicules, somewhat coarser at both ends. The distinct girdle fringe has longer and coarser spicules which are whitish with small orange-brown patches. The foot, somewhat narrow and yellowish-white, is in the center of the ventral area, the hypnotum being a light grayish-tan.

Both specimens compare quite well with the original description and valve illustrations (Figures 14–17). The short, rather blunt spicules exhibit weak, vertical striations, as also mentioned in the description (Figure 18). However, the holotype (Figure 19) is larger with the extended length estimated at 33 mm, and has an elongate-oval shape with roundly-pointed extremities. Although the anterior of the dissected specimen is narrower, gradually widening to the posterior end, the shape of this specimen may be an artefact of preservation. Also, the tail valve of the Korean specimens is somewhat less pointed (Fig, 17). The color is somewhat different, the girdle of the holotype being reddish brown, while the dissected specimen has a purplish tinge and the second speeimen is orange-brown. Additionally, the original description mentions a unicolored girdle, while the girdles of both Jeju specimens exhibit some mottling. Coloration in chiton species, often in the girdle, can be quite variable. Of the two specimens obtained, the smaller one resembles more closely the specimen figured in Saito (2000); however, the head valve in the illustration is more prominent.

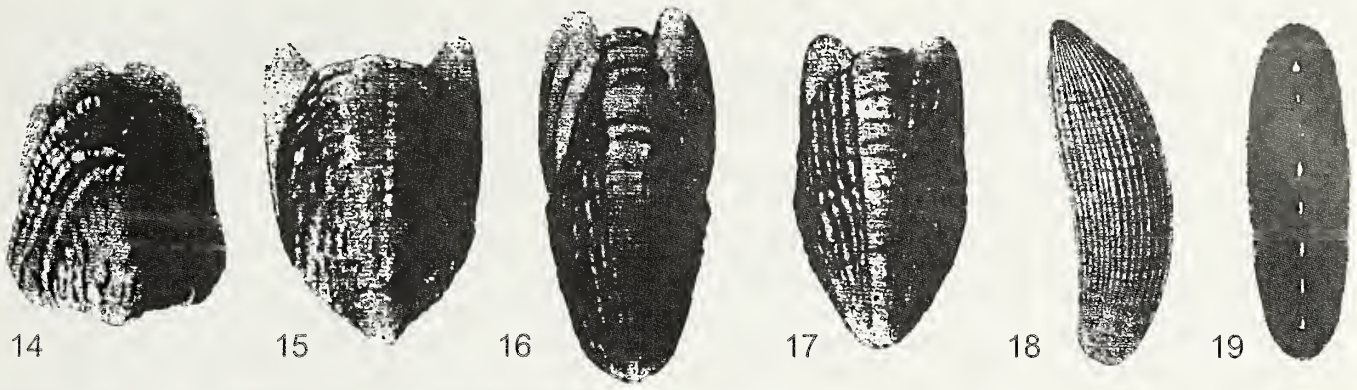
Distribution: South-central Japan to Jeju Island, southwestern South Korea.

DISCUSSION

Van Belle (1983; see also Kaas and Van Belle, 1998) and others have placed Cryptoplax in the subfamily Cryptoplacinae within Acanthochitonidae, but Sirenko (2006; see also Schwabe, 2014) has more recently accepted the same grouping as a family, Cryptoplacidae. Chitons belonging to *C. propior* are apparently much smaller than other adult members of this genus, and previously reported only from Izu-Oshima Island, south of Tokyo, eastern Japan, and the type locality, the Shima Peninsula, in east-central Japan, (Is. and Iw. Taki, 1930). Although the species has been known for the Japanese fauna for more than 80 years, surprisingly little is known about its distribution, ecology, and life cycle. Is. Taki, in various studies on Japanese Polyplacophora, made reference to it (1938; 1961; 1962). Saito (2000) included an illustration of a curled specimen, and Higo and Goto (1993) and Higo et al. (1999) included it in their listing of the Japanese Polyplacophora. Kaas and Van Belle (1998) also included this species in their catalogue of



Figures 12–13. *Cryptoplax propior.* SEM views of girdle and valves. **12.** Median area. **13.** Posterior area. Abbreviations: **s:** spicules; **f:** girdle fringe; **g:** granular rows.



Figures 14–19. *Cryptoplax propior.* Original illustrations. **14.** Head valve. **15.** Second valve. **16.** Median valve. **17.** Tail valve. **18.** Girdle spicule. **19.** Holotype (dorsal view). No scale provided. (From Is. and Iw. Taki, 1930, Venus, The Japanese Journal of Malacology; used with permission.)

Recent chitons, as well as Saito in his discussion of the taxonomy of the genus *Cryptoplax* (Saito, 1994).

The original detailed description made no mention of the habitat of *C. propior* or the depth at which it was obtained; however, Saito (2000), stated that it is found "on the rhizoids of laminarian brown algae in the subtidal zone". Bergenhayn (1933) examined specimens from the intertidal zone at Misaki (Miura), Sagami Bay, which resembled those in the original description, but were smaller and could possibly be subadults. According to Bergenhayn, his speeimens agreed in the morphology of the tegmentum and radula with the original description but the tegmentum eolor was yellowish-white instead of brown (Bergenhayn, 1933). Leloup (1940) commented on Bergenhayn's specimens but provided no new details. Saito (2006) mentioned *C. propior* in his list of chitons from the Sagami Sea and questioned Bergenhayn's record, asserting that this species is usually found in the subtidal zone among rhizoids of laminarian algae. However, Higo et al. (1999), while including the Misaki reference, listed the habitat of this species as "intertidal, rocks and gravel", suggesting a less-restricted habitat.

Saito (2006) also stated that *C. propior* closely resembles juvenile specimens of *C. japonica*. According to Saito (2000), one of the characteristics that separates the two species is the presence of 'radiating rows of granules" on pleurolateral areas of *C. propior*. In correspondence, he also stated that the morphology of the larger spicules on the perinotum is an important feature in separating *C. propior* from *C. japonica*; these spicules are "very short, thick and rather blunt at the tip", while those of *C. japonica* are long, slender, and pointed (Saito, pers. comm.).

SEM photos of the median and posterior areas of the perinotum of the Jeju specimens (Figures 12, 13) show spicules that resemble Saito's diagnosis, as well as the radiating rows of granules on the valves (Figure 12). Furthermore, SEM photos of *C. propior* provided by Saito compare well with those of specimens from Jeju Island (Saito, pers. comm.). In contrast, Hong et al. (1999), in their deseription of *Cryptoplax japonica*, mention the larger perinotum spicules as being slightly curved, smooth, and sharply pointed at the tip, differing from the short, thick, blunt, often striated spicules of the specimens in this study. An examination of the valves and girdle spicules of juvenile and subadult specimens of *Cryptoplax* specimens obtained from the east coast of the island, revealed a coarser sculpture of fewer radiating granular ribs and long, slender, pointed spicules characteristic of *C. japonica*, thus confirming the presence of two distinct species on the island.

CONCLUSION

Cryptoplax propior has both a restricted distribution and habitat in Japan. It has now been obtained from two widely-separated localities in Jeju Island, and may have a wider distribution there. This species and *C. japonica* are the northernmost representatives of the genus *Cryptoplax*, with a relatively restricted range, being reported from the northwestern Pacific, mainly from Korea and Japan.

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We would like to thank Bruno Anseeuw for literature and correspondence with Hiroshi Saito, who provided valuable information and illustrations, as well as Jan Haspeslagh, who supplied the original deseription and illustration of *C. propior*. Enrico Schwabe made available many references, as well as a translation of Bergenhayn's comments. Thanks also go to Konstantin Lutaenko, who provided literature, Doug Eernisse, who reviewed the manuscript, and provided literature and many valuable suggestions for improvement, and a second reviewer who also gave helpful advice. Dr. K. Hasegawa, editor of *The Venus*, gave permission to use the figures of *C. propior* from the original description. This study was supported by National Fisheries Research and Development Institute of Korea (NFRDI RP-2013-FR-088).

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Notice

Florida United Malacologists (FUM) 2015 – First Announcement

I am pleased to announce that the sixth annual meeting of Florida United Malacologists (FUM) will take place on Saturday, February 28, 2015, at The Florida Museum of Natural History, University of Florida, in Gainesville. This one-day meeting brings together anyone with an interest in mollusks to discuss a wide variety of topics including but not limited to biology, paleontology, archaeology, ecology, and conservation of mollusks. This relaxed and informal venue offers an excellent chance for students, amateurs, naturalists, citizen scientists, and professionals to meet and share ideas.

If you are interested in giving a presentation please submit a brief abstract of 150 words or less by February 1, 2015. The gathering is free and box lunches and dinner at a local restaurant (to be arranged) will be available at cost to participants. Parking is free, as are most museum exhibits. Behind-the-scenes visits to the FLMNH collection at Dickinson Hall about a mile away can be arranged for the week before or after the meeting.

Please send inquiries and reservations to John Slapcinsky at slapcin@flmnh.ufl.edu. The deadline for submission of abstracts is February 1, 2015. The FUM program, abstracts, and times of presentations will be posted on the FLMNH web site, http://www.flmnh.ufl.edu/fum2015/home/ shortly after the abstract submission deadline.

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THE NAUTILUS publishes articles on all aspects of the biology, paleontology, and systematics of mollusks. Manuscripts describing original, unpublished research and review articles will be considered. Brief articles, not exceeding 1000 words, will be published as notes and do not require an abstract. Notices of interest to the malacological community will appear in a notices section.

Manuscripts: Each original manuscript and accompanying illustrations should be submitted to the editor preferably via e-mail or as hardcopy in triplicate.

Text must conform to the dimensions of 8½ × 11-inch paper, double-spaced, and single-column throughout (including literature cited, tables, and figure captions). Authors should follow the general recommendations of *Scientific Style and Format—The CSE Manual for Authors, Editors, and Publishers*, available from the Council of Science Editors at www.councilscienceeditors.org. The first mention of a scientific name in the text should be accompanied by the taxonomic authority, including year. Latinized names and other words to be printed in italics must be underlined; leave other formatting indications to the editor. Metric, not English, units are to be used. Geochronologic modifiers should be capitalized only when units are formally recognized: for instance, use Late Cretaceous but early Miocene. Likewise, only modifiers of formally recognized chronostratigraphic units are capitalized: use Lower Jurassic but upper Oligocene.

The sequence of sections should be title page, abstract, introduction, materials and methods, results, discussion, acknowledgments, literature cited, tables, figure captions, figures. The title page should include the title, author's name(s) and address(es). If corresponding author is not the senior author, please indicate. The abstract should summarize in 250 words or less the scope, main results, and conclusions of the article. Abstracts should be followed by a list of additional key words. All references cited in the text must appear in the Literature Cited section and vice-versa. Please follow a recent issue of THE NAUTILUS for bibliographic style, noting that journal titles must be unabbreviated. Information on plates and figures should be cited only if not included within the pagination of cited work. Tables must be numbered and each placed on a separate page. If in doubt, please follow a recent issue of the journal for sequence of sections and other style requirements.

Illustrations: Illustrations are rendered either at full-page width (maximum width 17 cm) or column width (maximum width 8.2 cm). Please take these dimensions into consideration when preparing illustrations. Page-width illustrations ideally should span the entire width of printed page (17 cm). "Tall" page-width illustrations should be avoided, square or "landscape" formats work better. Please design plates accordingly, such that there will be enough space left at the bottom of printed page for plate caption. (Digital technology has made this task much easier.)

All line drawings must be in black, clearly detailed, and completely labeled. Abbreviation definitions must be included in the caption. Line drawings must be high resolution files at at least 600 dpi (dots per inch) resolution at actual size. Standard digital formats for line drawings include .tif, .bmp, .psd, .eps, and .pdf.

Photographs may be submitted in black-and-white or color, preferably in RGB mode if in color. Standard digital formats for photographs include .tif, .psd, .jpg, or .pdf. Photographs must be high resolution files at least 300 dpi resolution at actual size.

If more than one figure is included in an illustration, all figures are to be consecutively numbered (Figures 1, 2, 3, . . . , NOT Figures 1A, 1B, 1C, . . . , NOR Plate 1, Figure 1, . . .). In illustrations with more than one figure, make sure that blank areas between figures is kept to a minimum, thereby allowing for more area for each individual figure.

Compressed files (e.g., .jpg) may be used to facilitate transmission of files during original submission, but may not be acceptable at final submission (see below).

Voucher Specimens: Deposition of the holotype in a recognized institutional, public collection is a requirement for publication of articles in which new species-level taxa are described. Deposition of paratypes in institutional collections is strongly encouraged, as is the deposition of representative voucher specimens for all other types of research work.

The Editorial Process: Upon receipt, all manuscripts are assigned a number and acknowledged. The editor reserves the right to return manuscripts that are substandard or not appropriate in scope for THE NAUTILUS. Manuscripts deemed appropriate for the journal will be sent for critical review to at least two reviewers. The reviewers' recommendations will serve as basis for rejection or continuation of the editorial process. Reviewed manuscripts will be sent back to authors for consideration of the reviewers' comments. The revised version of the manuscript may at this point be considered accepted for publication by the journal.

Final Submission: Authors of accepted manuscripts are required to submit a final version via e-mail to the editor at jleal@shellmuseum.org. Please do not send low-resolution or compressed illustration files at this stage. Send any files larger than 20 Mb on a CD or DVD to the editor.

Proofs: After typesetting, proofs will be sent to the author. Author should read proofs carefully and send corrections to the editor within 48 hours. Changes other than typesetting errors will be charged to the author at cost.

Offprints: An order form for offprints will accompany the proofs. Offprints will be ordered through the editor. Authors with institutional, grant, or other research support will be asked to pay for page charges at the rate of $60 per page.